F ENT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

REINHOLD COHN AND PARTNERS
P.O. Box 4060
61040 Tel Aviv
ISRAËL

Date of mailing (day/month/year)	
08 December 2000 (08.12.00)	
Applicant's or agent's file reference	**IMPORTANT NOTIFICATION**
123334.5 MM	
International application No.	**International filing date** (day/month/year)
PCT/IL00/00184	24 March 2000 (24.03.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
SHINITZKY, Meir Derech Haganim Street 20 46910 Kfar Shmari Yahu israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
SHINITZKY, Meir Derech Haganim 20 46910 Kfar Shmaryahu Israel	IL	IL
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
123334.5 MM		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/IL 00/00184	24/03/2000	25/03/1999
Applicant		
YEDA RESEARCH AND DEVELOPMENT CO.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 30,35,37,38,40,42 relate to the use of the compounds of formula I in the treatment of diseases which actually are not well defined. The use of the definitions "for inducing/can be treated by phosphorylation of intracellular proteins", "for promotion of cell differentiation", "for induction of hormone-like signaling", "for the treatment of disorders in which HGH is involved" and "for the treatment of disorders involving EGF" in the present context is considered to lead to a lack of clarity within the meaning of Article 6 PCT. The lack of clarity is such as to render a meaningful complete search impossible. Furthermore, the term "analogs" used in claim 39 is vague and unclear and leaves in doubt as to the meaning of the technical features (substances) to which it refers. Again the lack of clarity is such as to render a meaningful complete search impossible.
Consequently, the search has been restricted to the compounds of the general formula I and to the diseases mentioned in claims 20-23,25,31-34,36,41 with due regard to the general idea underlying the present invention.

Claims searched completely: 1-29,31-34,36,41,43,44
Claims searched incompletely: 30,35,37-40,42

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 00/00184

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/665 A61P43/00 A61P35/00 A61P35/02 A61P3/10
C07F9/6574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 09139 A (ALLELIX BIOPHARMA ;BEGLEITER LEATH E (CA); WICKENS PHILIP L (CA);) 24 February 2000 (2000-02-24) abstract page 11, line 11 -page 12, line 10 page 13, line 26 -page 14, line 22; claims; example 1 ---	1-4, 18-28, 30,35, 37,38, 40,42,43
P,X	MUKAI, MUTSUKO ET AL: "Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA)" INT. J. CANCER (1999), 81(6), 918-922 , XP000949280 the whole document --- -/--	1-4, 18-28, 30-35, 37,38, 40-43

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 2000	16/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hoff, P

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) *DOCUMENTS CONSIDERED TO BE RELEVANT*

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Tumor metastasis inhibitors containing 1-O-acylglycerol-2,3-phosphates" retrieved from STN Database accession no. 126:220705 XP002148570 abstract & JP 09 025235 A (SAGAMI CHEM RES, JAPAN) 28 January 1997 (1997-01-28) ---	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506 XP002148571 abstract & JP 07 258278 A (SAGAMI CHEM RES, JAPAN) 9 October 1995 (1995-10-09) ---	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Method for preparation of 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:144502 XP002148572 abstract & JP 06 228169 A (SAGAMI CHEM RES, JAPAN) 16 August 1994 (1994-08-16) ---	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Promoters of protein phosphokinase C activation containing 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:350234 XP002148573 abstract & JP 07 149772 A (SAGAMI CHEM RES, JAPAN) 13 June 1995 (1995-06-13) --- -/--	1-4, 18-28, 30, 35-40, 42,43

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 565 439 A (PIAZZA GARY A ET AL) 15 October 1996 (1996-10-15) abstract column 1, line 60 -column 2, line 39; claims; example II	1-4, 18-27, 30-35, 37,38, 40-42
X	D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300 see compounds (VI) and (VII) pages 1111 and 1114	1,5,7, 13,18, 19,24, 28,29
X	REVEL, MONIQUE ET AL: "Phosphorus heterocycles. XXVII. NMR study of 4-monosubstituted 1,3,2-dioxa- and -dithiaphospholane derivatives" ORG. MAGN. RESON. (1976), 8(8), 399-406 , XP000949315 page 399	1,5,7, 18,19, 24,28,29
X	SHINITZKY M ET AL: "Formation of 1,3-cyclic glycerophosphate by the action of phospholipase C on phosphatidylglycerol." JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 JUL 5) 268 (19) 14109-15. , XP000946147 figures 6,10	1,12,14, 16,18, 19,24, 28,29
X	T. UKITA ET AL.: "Organic Phosphates. I. Synthesis of 1,2-Diol Cyclic Phosphates." PHARM. BULL., vol. 5, 1957, pages 121-126, XP000949388 see compounds (I) and (V) pages 122-124	1,6,8, 18,19,24
X	SU, BANGYING ET AL: "Identification of a putative tumor marker in breast and colon cancer" CANCER RES. (1993), 53(8), 1751-4 , XP000946184 figure 4	1,8,18, 19,24
X	ABBOTT, STEVEN J. ET AL: "Chiral '160, 170, 180!phosphate monoesters. 1. Asymmetric synthesis and stereochemical analysis of '1(R)-160, 170, 180!phospho-(S)-propane-1,2-diol" J. AM. CHEM. SOC. (1978), 100(8), 2558-60 , XP000946182 see scheme II page 2558	1,6,18, 19,24

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 00/00184

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0009139 A	24-02-2000	AU 5473599 A	06-03-2000
JP 9025235 A	28-01-1997	NONE	
JP 7258278 A	09-10-1995	NONE	
JP 6228169 A	16-08-1994	NONE	
JP 7149772 A	13-06-1995	NONE	
US 5565439 A	15-10-1996	NONE	

PATENT COOPERATION TREATY

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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
123334.5 MM		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00184	24/03/2000	25/03/1999
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23/10/2000	07.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Hoff, P Telephone No. +31 70 340 3520

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00184

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-36	as originally filed				

Claims, No.:

1-43	as received on	05/03/2001	with letter of	05/03/2001	

Drawings, sheets:

1/9-9/9	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 25-35 with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. 25-35 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 5-10,24,43

	No:	Claims	1-4,11-23,25-42
Inventive step (IS)	Yes:	Claims	5-10,24,43
	No:	Claims	1-4,11-23,25-42
Industrial applicability (IA)	Yes:	Claims	see Separate Sheet
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the report

In an attempt to overcome the novelty objections raised in the light of the disclosure of D1 to D11, the Applicant has filed in his letter dated 05.03.01, amended claims which comprise features in the form of a so-called disclaimer intended to exclude subject-matter from the said prior art documents.

However, it would be allowable under Article 34(2)(b) PCT to formulate a disclaimer which is precisely defined and limited to the prior art disclosure, provided this disclosure is an accidental novelty-destroying disclosure. A disclaimer is only allowable if the cited document containing said disclosure has no relevance for any further examination of the claimed invention and it must then disappear from the prior art field to be taken into consideration.

In the present case, D1 to D5 undisputedly relate to the same field as that of the claimed invention. The fact that they deal with the drugs of the claimed invention (cyclic glycerophosphates and analogs thereof) and their use for the same therapeutic indications (cancer and diabetes) makes D1 to D5 relevant with or without the disclaimer (a) in claims 1 and 13. Accordingly, said disclaimer (a) ("when Y is -(CH2)m-, m=0, and R is H or cation, X is not CH2Oacyl") is unacceptable under Article 34(2)(b) PCT.

Since some of the amendments made in claims 1 and 13 have been considered to go beyond the disclosure as filed (Rule 70.2(c)), this international preliminary examination report has been established as if said amendments have not been made (i.e. without the disclaimer "when Y is -(CH2)m-, m=0, and R is H or cation, X is not CH2Oacyl" in claims 1 and 13).

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 25-35 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Tumor metastasis inhibitors containing 1-O-acylglycerol-2,3-phosphates" retrieved from STN Database accession no. 126:220705

D2: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506

D3: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Method for preparation of 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:144502

D4: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Promoters of protein phosphokinase C activation containing 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:350234

D5: US 5 565 439 A (PIAZZA GARY A ET AL) 15 October 1996 (1996-10-15)

D6: D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300

D7: REVEL, MONIQUE ET AL: "Phosphorus heterocycles. XXVII. NMR study of 4-monosubstituted 1,3,2-dioxa- and -dithiaphospholane derivatives" ORG. MAGN. RESON. (1976), 8(8), 399-406 , XP000949315

D8: SHINITZKY M ET AL: "Formation of 1,3-cyclic glycerophosphate by the action of phospholipase C on phosphatidylglycerol." JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 JUL 5) 268 (19) 14109-15. , XP000946147

D9: T. UKITA ET AL.: "Organic Phosphates. I. Synthesis of 1,2-Diol Cyclic Phosphates." PHARM. BULL., vol. 5, 1957, pages 121-126, XP000949388

D10: SU, BANGYING ET AL: "Identification of a putative tumor marker in breast and colon

cancer" CANCER RES. (1993), 53(8), 1751-4 , XP000946184
D11: ABBOTT, STEVEN J. ET AL: "Chiral [16O, 17O, 18O]phosphate monoesters. 1. Asymmetric synthesis and stereochemical analysis of [1(R)-16O, 17O, 18O]phospho-(S)-propane-1,2-diol" J. AM. CHEM. SOC. (1978), 100(8), 2558-60 , XP000946182

It is pointed out that the document "MUKAI, MUTSUKO ET AL: "Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA)" INT. J. CANCER (6/1999), 81(6), 918-922 , XP000949280 " could be relevant for novelty and inventive step for the subject-matter of claims 1-4,11-23,25-32,34,35,38-42 if the priority of the pending application, which has not been checked is considered as invalid.

1.Claims 1-21,25-35,38-43 involve compositions or substances in a method of treatment of the human/animal body.
For the assessment of the present claims 1-21,25-35,38-43 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2. Documents D1, D2 and D3 disclose pharmaceutical compositions comprising the compounds of general formula I (Y is (CH2)m with m=0; X is CH2Oacyl) for use in the treatment of tumor and metastasis.
The attention of the Applicant is drawn to the fact, that present claims 1-4,11-21 relate to a pharmaceutical composition per se comprising cyclic glycerophosphates of formula I, and will be anticipated by any disclosure describing the compounds of formula I as medicaments, regardless of the intended therapeutic use.
It is also pointed out, that the mere explanation of an effect obtained when using a compound in a known process, even if the explanation relates to a pharmaceutical effect which was not known for that compound, cannot confer novelty to said process. In the present case, the newly discovered technical effect of inducing phosphorylation in intracellular proteins or promoting cell differentiation does not confer novelty on the claims

directed to the use of a cyclic glycerophosphate of formula I for a known purpose (treatment of malignant diseases). No novelty exists, if the claim is directed to the use of a known compound for a known purpose, even if a newly discovered technical effect (inducing phosphorylation in intracellular proteins/promoting cell differentiation) underlying said known use is indicated in that claim.
The same applies to claims 31,32,34,35 and 40, which relate to vague and unclear disorders which leave the reader in doubt as to the meaning of the real diseases to which they refer (see also Section VIII). Accordingly, said claims will be considered as including also malignant disorders.
Therefore, in view of D1,D2 and D3 the subject-matter of claims 1-4,11-23,25-32,34,35,38-42 cannot be regarded as being novel and does not meet the requirements of Article 33(2) PCT.

3. Moreover, the document D4 discloses the compounds of formula I as promoter of protein phosphokinase C activation (thereby inducing phosphorylation in intracellular proteins), useful in the treatment of diabetes. Document D5 describes lysophosphatidic acid derivatives of formula I for treating hyperproliferative disorders, such as cancers.
Taking into account the remarks made in point 2 above, the lack of novelty in sense of Article 33(2) PCT is further emphasized by the disclosure of documents D4 and D5 as follows:
-lack of novelty of claims 1-4,11-23,25,26,31-38,40-42 with regard to D4
-lack of novelty of claims 1-4,11-22,25-27,31,32,34,35,38-41 with regard to D5

4. Furthermore, D6 discloses the compounds phenyl 1,3-cyclic propanediol phosphate and phenyl 1,2-cyclic propanediol phosphate (see page 1111); D7 discloses phenyl 1,2-cyclic propanediol phosphate (see page 399); D8 discloses the compounds cyclic dihydroxyacetone phosphate, 1,3-cyclic propanediol phosphate and 1,3-cyclic glycerophosphate (see Fig.6 and 10); D9 discloses 1,2-cyclic propanediol phosphate and 1,2-cyclic glycerophosphate (see page 122); D10 discloses 1,2-cyclic glycerophosphate (see Fig.4); D11 discloses the compound 1,2-cyclic propanediol phosphate (see Scheme II, page 2558).
The attention of the Applicant is drawn to the fact that claim 18 relates, with regard to the obligatory features, to a (pharmaceutical) composition containing the cyclic glycerophosphates of formula I with a non-defined carrier. Only the claims 13-16 and 19-21 relate clearly to a composition for the first use in therapy.

It is pointed out that in a claim directed to a composition of a known structurally defined active agent with at least one auxiliary substance, in which the feature "a pharmaceutically acceptable carrier" means that something is added to the active agent, the admixture of an unspecified carrier cannot, in view of the unlimited number of substances which may enter into consideration, be deemed a substance and distinctive addition to the active agent. Accordingly, the mere statement that a non- defined carrier of unspecified effect should be mixed with the compound of formula I, albeit itself defined as such, cannot be used under Article 33(2) PCT as a delimiting feature in the said product claim.
Therefore, the lack of novelty of claim 18 in sense of Article 33(2) PCT is further emphasized by the disclosure of documents D6 to D11.

5. It seems however, that the subject-matter of claims 5-10,24,43 appears to be novel and inventive meeting thus the requirements of Articles 33(2) and 33(3) PCT.
5.1. None of the prior art documents discloses the cyclic glycerophosphate derivatives of those claims and their use in therapy.
5.2. In the light of the prior art, the problem to be solved can be regarded as how to provide compounds for inducing phosphorylation in intracellular proteins of target cells, useful in the prevention and treatment of various diseases (such as malignant disorders or NIDDM).
No indications were found that would have led the skilled person to synthesize these compounds in order to solve the problem posed, so an inventive step in the sense of Article 33(3) PCT is acknowledged.

Re Item VI
Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO-A-00/09139	24.02.00	10.08.99	10.08.98

The documents WO-A-0009139 which describes a pharmaceutical composition for use in therapy comprising the cyclic glycerophosphates of formula I is relevant for novelty for the subject-matter of claims 1-4,11-23,25,26,31,32,34,35,38,40-42.The priorities of the conflicting and the pending applications have however not been checked.

Re Item VII

Certain defects in the international application

1. The expression "a therapeutically effective amount" used in claims 25,27,31 is superfluous and does not comply with Rule 9.1.(iv) PCT.

2. The term "non-limiting" (page 16, line 11) does not comply with Rule 9.1.(iv) PCT.

3. The abbreviations "RT" (page 19), "HBSS" (page 20), "ECL" and "MBP" (page 21), "MEK" (page 22), "ERK" and "IP" (page 25), "DAB" (page 32) are not specified as required under Rule 10.1.(e) PCT.

4. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 to D5 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

1. Claims 11,12,17,18,20,21,25,26,31,32,34,35,38,40 relate to a composition comprising- and to the use of- a compound of formula I for treating a disease which actually is not well defined. Treatment of "disorders which can be treated by phosphorylation of intracellular proteins", of "diseases involving hormone-like signalling", of "diseases in which HGH/EGF are involved" and of "diseases involving cell differentiation" are not clear definitions of diseases rendering thus the scope of the protection of said claims obscure (Article 6 PCT). It is pointed out that the mechanism of action of a drug cannot be considered in itself as a therapeutic application; the discovery that a substance has a particular pharmacological profile still needs to find a practical application in the form of a defined, real treatment of

a pathological condition.

2. The term "analogs" used in claim 36 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features (substances) to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

3. The terms "alkyl", "acyl" and "optionally substituted aryl" as given in claim 1 obscure the scope of protection and have not been made clear and consistent with the definitions given in claim 2 (Article 6 PCT).

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
123334.5 MM		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL00/00184	24/03/2000	25/03/1999
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23/10/2000	07.08.2001
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Hoff, P Telephone No. +31 70 340 3520

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/IL00/00184

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-36	as originally filed

Claims, No.:

1-43	as received on	05/03/2001	with letter of	05/03/2001

Drawings, sheets:

1/9-9/9	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 25-35 with respect to industrial applicability.

because:

   ☒ the said international application, or the said claims Nos. 25-35 with respect to industrial applicability relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 5-10,24,43

	No:	Claims	1-4,11-23,25-42
Inventive step (IS)	Yes:	Claims	5-10,24,43
	No:	Claims	1-4,11-23,25-42
Industrial applicability (IA)	Yes:	Claims	see Separate Sheet
	No:	Claims	

2. Citations and explanations
see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item I

Basis of the report

In an attempt to overcome the novelty objections raised in the light of the disclosure of D1 to D11, the Applicant has filed in his letter dated 05.03.01, amended claims which comprise features in the form of a so-called disclaimer intended to exclude subject-matter from the said prior art documents.

However, it would be allowable under Article 34(2)(b) PCT to formulate a disclaimer which is precisely defined and limited to the prior art disclosure, provided this disclosure is an accidental novelty-destroying disclosure. A disclaimer is only allowable if the cited document containing said disclosure has no relevance for any further examination of the claimed invention and it must then disappear from the prior art field to be taken into consideration.

In the present case, D1 to D5 undisputedly relate to the same field as that of the claimed invention. The fact that they deal with the drugs of the claimed invention (cyclic glycerophosphates and analogs thereof) and their use for the same therapeutic indications (cancer and diabetes) makes D1 to D5 relevant with or without the disclaimer (a) in claims 1 and 13. Accordingly, said disclaimer (a) ("when Y is -(CH2)m-, m=0, and R is H or cation, X is not CH2Oacyl") is unacceptable under Article 34(2)(b) PCT.

Since some of the amendments made in claims 1 and 13 have been considered to go beyond the disclosure as filed (Rule 70.2(c)), this international preliminary examination report has been established as if said amendments have not been made (i.e. without the disclaimer "when Y is -(CH2)m-, m=0, and R is H or cation, X is not CH2Oacyl" in claims 1 and 13).

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 25-35 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Tumor metastasis inhibitors containing 1-O-acylglycerol-2,3-phosphates" retrieved from STN Database accession no. 126:220705

D2: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506

D3: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Method for preparation of 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:144502

D4: DATABASE CHEMABS [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Promoters of protein phosphokinase C activation containing 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:350234

D5: US 5 565 439 A (PIAZZA GARY A ET AL) 15 October 1996 (1996-10-15)

D6: D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300

D7: REVEL, MONIQUE ET AL: "Phosphorus heterocycles. XXVII. NMR study of 4-monosubstituted 1,3,2-dioxa- and -dithiaphospholane derivatives" ORG. MAGN. RESON. (1976), 8(8), 399-406 , XP000949315

D8: SHINITZKY M ET AL: "Formation of 1,3-cyclic glycerophosphate by the action of phospholipase C on phosphatidylglycerol." JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 JUL 5) 268 (19) 14109-15. , XP000946147

D9: T. UKITA ET AL.: "Organic Phosphates. I. Synthesis of 1,2-Diol Cyclic Phosphates." PHARM. BULL., vol. 5, 1957, pages 121-126, XP000949388

D10: SU, BANGYING ET AL: "Identification of a putative tumor marker in breast and colon

cancer" CANCER RES. (1993), 53(8), 1751-4 , XP000946184
D11: ABBOTT, STEVEN J. ET AL: "Chiral [16O, 17O, 18O]phosphate monoesters. 1. Asymmetric synthesis and stereochemical analysis of [1(R)-16O, 17O, 18O]phospho-(S)-propane-1,2-diol" J. AM. CHEM. SOC. (1978), 100(8), 2558-60 , XP000946182

It is pointed out that the document "MUKAI, MUTSUKO ET AL: "Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA)" INT. J. CANCER (6/1999), 81(6), 918-922 , XP000949280 " could be relevant for novelty and inventive step for the subject-matter of claims 1-4,11-23,25-32,34,35,38-42 if the priority of the pending application, which has not been checked is considered as invalid.

1.Claims 1-21,25-35,38-43 involve compositions or substances in a method of treatment of the human/animal body.
For the assessment of the present claims 1-21,25-35,38-43 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2. Documents D1, D2 and D3 disclose pharmaceutical compositions comprising the compounds of general formula I (Y is (CH2)m with m=0; X is CH2Oacyl) for use in the treatment of tumor and metastasis.
The attention of the Applicant is drawn to the fact, that present claims 1-4,11-21 relate to a pharmaceutical composition per se comprising cyclic glycerophosphates of formula I, and will be anticipated by any disclosure describing the compounds of formula I as medicaments, regardless of the intended therapeutic use.
It is also pointed out, that the mere explanation of an effect obtained when using a compound in a known process, even if the explanation relates to a pharmaceutical effect which was not known for that compound, cannot confer novelty to said process. In the present case, the newly discovered technical effect of inducing phosphorylation in intracellular proteins or promoting cell differentiation does not confer novelty on the claims

directed to the use of a cyclic glycerophosphate of formula I for a known purpose (treatment of malignant diseases). No novelty exists, if the claim is directed to the use of a known compound for a known purpose, even if a newly discovered technical effect (inducing phosphorylation in intracellular proteins/promoting cell differentiation) underlying said known use is indicated in that claim.
The same applies to claims 31,32,34,35 and 40, which relate to vague and unclear disorders which leave the reader in doubt as to the meaning of the real diseases to which they refer (see also Section VIII). Accordingly, said claims will be considered as including also malignant disorders.
Therefore, in view of D1,D2 and D3 the subject-matter of claims 1-4,11-23,25-32,34,35,38-42 cannot be regarded as being novel and does not meet the requirements of Article 33(2) PCT.

3. Moreover, the document D4 discloses the compounds of formula I as promoter of protein phosphokinase C activation (thereby inducing phosphorylation in intracellular proteins), useful in the treatment of diabetes. Document D5 describes lysophosphatidic acid derivatives of formula I for treating hyperproliferative disorders, such as cancers.
Taking into account the remarks made in point 2 above, the lack of novelty in sense of Article 33(2) PCT is further emphasized by the disclosure of documents D4 and D5 as follows:
-lack of novelty of claims 1-4,11-23,25,26,31-38,40-42 with regard to D4
-lack of novelty of claims 1-4,11-22,25-27,31,32,34,35,38-41 with regard to D5

4. Furthermore, D6 discloses the compounds phenyl 1,3-cyclic propanediol phosphate and phenyl 1,2-cyclic propanediol phosphate (see page 1111); D7 discloses phenyl 1,2-cyclic propanediol phosphate (see page 399); D8 discloses the compounds cyclic dihydroxyacetone phosphate, 1,3-cyclic propanediol phosphate and 1,3-cyclic glycerophosphate (see Fig.6 and 10); D9 discloses 1,2-cyclic propanediol phosphate and 1,2-cyclic glycerophosphate (see page 122); D10 discloses 1,2-cyclic glycerophosphate (see Fig.4); D11 discloses the compound 1,2-cyclic propanediol phosphate (see Scheme II, page 2558).
The attention of the Applicant is drawn to the fact that claim 18 relates, with regard to the obligatory features, to a (pharmaceutical) composition containing the cyclic glycerophosphates of formula I with a non-defined carrier. Only the claims 13-16 and 19-21 relate clearly to a composition for the first use in therapy.

It is pointed out that in a claim directed to a composition of a known structurally defined active agent with at least one auxiliary substance, in which the feature "a pharmaceutically acceptable carrier" means that something is added to the active agent, the admixture of an unspecified carrier cannot, in view of the unlimited number of substances which may enter into consideration, be deemed a substance and distinctive addition to the active agent. Accordingly, the mere statement that a non- defined carrier of unspecified effect should be mixed with the compound of formula I, albeit itself defined as such, cannot be used under Article 33(2) PCT as a delimiting feature in the said product claim.
Therefore, the lack of novelty of claim 18 in sense of Article 33(2) PCT is further emphasized by the disclosure of documents D6 to D11.

5. It seems however, that the subject-matter of claims 5-10,24,43 appears to be novel and inventive meeting thus the requirements of Articles 33(2) and 33(3) PCT.
5.1. None of the prior art documents discloses the cyclic glycerophosphate derivatives of those claims and their use in therapy.
5.2. In the light of the prior art, the problem to be solved can be regarded as how to provide compounds for inducing phosphorylation in intracellular proteins of target cells, useful in the prevention and treatment of various diseases (such as malignant disorders or NIDDM).
No indications were found that would have led the skilled person to synthesize these compounds in order to solve the problem posed, so an inventive step in the sense of Article 33(3) PCT is acknowledged.

Re Item VI

Certain documents cited

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO-A-00/09139	24.02.00	10.08.99	10.08.98

The documents WO-A-0009139 which describes a pharmaceutical composition for use in therapy comprising the cyclic glycerophosphates of formula I is relevant for novelty for the subject-matter of claims 1-4,11-23,25,26,31,32,34,35,38,40-42.The priorities of the conflicting and the pending applications have however not been checked.

Re Item VII

Certain defects in the international application

1. The expression "a therapeutically effective amount" used in claims 25,27,31 is superfluous and does not comply with Rule 9.1.(iv) PCT.

2. The term "non-limiting" (page 16, line 11) does not comply with Rule 9.1.(iv) PCT.

3. The abbreviations "RT" (page 19), "HBSS" (page 20), "ECL" and "MBP" (page 21), "MEK" (page 22), "ERK" and "IP" (page 25), "DAB" (page 32) are not specified as required under Rule 10.1.(e) PCT.

4. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 to D5 is not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

1. Claims 11,12,17,18,20,21,25,26,31,32,34,35,38,40 relate to a composition comprising- and to the use of- a compound of formula I for treating a disease which actually is not well defined. Treatment of "disorders which can be treated by phosphorylation of intracellular proteins", of "diseases involving hormone-like signalling", of "diseases in which HGH/EGF are involved" and of "diseases involving cell differentiation" are not clear definitions of diseases rendering thus the scope of the protection of said claims obscure (Article 6 PCT). It is pointed out that the mechanism of action of a drug cannot be considered in itself as a therapeutic application; the discovery that a substance has a particular pharmacological profile still needs to find a practical application in the form of a defined, real treatment of

a pathological condition.

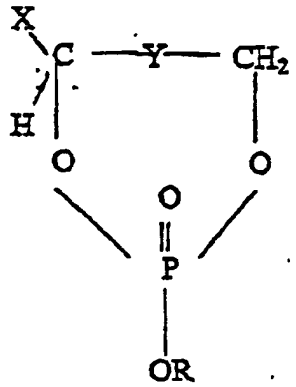
2. The term "analogs" used in claim 36 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features (substances) to which it refers, thereby rendering the definition of the subject-matter of said claim unclear (Article 6 PCT).

3. The terms "alkyl", "acyl" and "optionally substituted aryl" as given in claim 1 obscure the scope of protection and have not been made clear and consistent with the definitions given in claim 2 (Article 6 PCT).

ARTICLE84

CLAIMS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general formula I:



wherein

Y is $-(CH_2)_m-$, $-CH(OH)-$ or $-C(=O)-$, and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl; provided that:

(a) When Y is $-(CH_2)_m-$, m=0, and R is H or cation, X is not CH_2Oacyl; and

(b) Said compound is not one of

(i) Pheny 1,3-cyclic propanediol phosphate,

(ii) Pheny 1,2-cyclic propanediol phosphate,

(iii) Cyclic dihydroxyacetone phosphate,

(iv) 1,3,-cyclic propanediol phosphate

(v) 1,3-cyclic glycerophosphate,

(vi) 1,2-cyclic propanediol phosphate,

(vii) 1,2-cyclic glycerophosphate.

2. A pharmaceutical composition according to Claim 1, wherein said alkyl groups have 1-24 carbon atoms, said acyl groups are aliphatic saturated or

unsaturated C_1 - C_{24} acyl groups and said aryl group is a carbocyclic aryl group optionally substituted by C_1 - C_4 alkyl, halogen and/or hydroxy.

3. A pharmaceutical composition according to Claim 2, wherein said acyl groups are derived from natural fatty acids.

4. A pharmaceutical composition according to Claim 3, wherein said acyl group is a saturated aliphatic acyl group selected from acetyl, butyryl, caproyl, octanoyl, decanoyl, lauroyl, myristyl, palmitoyl and stearoyl, or an unsaturated aliphatic acyl group selected from palmitoleyl, oleyl, linoleyl, and ricinoleyl.

5. A pharmaceutical composition according to any one of Claims 1-4, wherein said aryl group is phenyl.

6. A pharmaceutical composition according to Claim 1, comprising phenyl 1,2-cyclic glycerophosphate.

7. A pharmaceutical composition according to Claim 1, comprising 3-acyl 1,2-cyclic glycerophosphate.

8. A pharmaceutical composition according to Claim 1, comprising cyclic oleyl lysophosphatidic acid.

9. A pharmaceutical composition according to Claim,1 comprising phenyl 1,3-cyclic glycerophosphate.

10. A pharmaceutical composition according to Claim1, comprising phenyl cyclic dihydroxyacetone phosphate.

11. A pharmaceutical composition for inducing phosphorylation in intracellular proteins of target cells comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of general Formula I of Claim 1.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general Formula I of Claim 1 for promotion of cell differentiation in target cells.

13. A pharmaceutical composition for the treatment of malignant diseases and disorders comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general Formula I of Claim 1 wherein

Y is $-(CH_2)_m-$, $-CH(OH)-$ or $-C(=O)-$, and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH2Oacyl or -CH2acyl; and

R is H, a cation, alkyl or optionally substituted aryl; provided that

when Y is $-(CH2)_m-$, m=0, and R is H or cation, X is not CH_2Oacyl.

14. A pharmaceutical composition according to Claim 13, wherein said malignant disorder is a blood malignancy.

15. A pharmaceutical composition according to Claim 14, wherein said blood malignancy is leukemia.

16. A pharmaceutical composition according to Claim 13, wherein said malignancy is breast cancer.

17. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound as defined in Claim 1, for induction of hormone-like signaling.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a as defined in Claim 13, for induction of hormone-like signaling wherein said hormone is selected from the group consisting of insulin, human growth hormone, and epidermal growth factor.

19. A pharmaceutical composition according to Claim 17 or 18 wherein said hormone is insulin and the composition is for the treatment of non-insulin-dependent diabetes mellitus (non-IDDM type II diabetes).

20. A pharmaceutical composition according to claim 17 or 18, wherein said hormone is human growth hormone (HGH) for the treatment of disorders in which HGH is involved.

21. A pharmaceutical composition according to Claim 17 or 18, wherein said hormone is epidermal growth factor (EGF) for the treatment of disorders involving EGF.

22. A compound as defined in claim 1.

23. A compound as defined in Claim 1, with the exception of the following compounds:

(i) compounds wherein Y is - $(CH_2)_m$ -, m is 0, X is CH_3, -CH_2OH or CH_2Oacyl wherein acyl is a saturated carboxylic acyl with more than 12 carbon atoms, and R is H or a cation;

(ii) compounds wherein Y is - $(CH_2)_m$ -, m is 1, X is H and R is H , a cation or phenyl; and

(iii) compounds wherein Y is – CH(OH) - , X is H and R is H, a cation or phenyl.

24. A compound according to Claim 29, selected from the group consisting of:

(i) phenyl 1,2 cyclic glycerophosphate;

(ii) phenyl cyclic dihydroxyacetone phosphate; and

(iii) cyclic oleyl lysophosphatidic acid.

25. A method for treatment of disorders and diseases which can be treated by phosphorylation of intracellular proteins comprising administering to the individual in need a therapeutically effective amount of a compound as defined in Claim 13.

26. A method according to claim 25 wherein said compound is a compound as defined in claim 1.

27. A method for the treatment of malignant diseases comprising administering to an individual in need a therapeutically effective amount of a compound as defined in claim 13.

28. A method according to Claim 27, wherein said malignant disease or disorder is blood malignancy.

29. A method according to Claim 28, wherein said blood malignancy is leukemia.

30. A method according to Claim 27, wherein said malignant disease is breast cancer.

31. A method for the treatment of diseases involving hormone-like signaling comprising administering to an individual in need a therapeutically effective amount of a compound as defined in Claim 13.

32. A method according to claim 31 wherein said compound is as defined in claim 1.

33. A method according to Claim 31 or 32, wherein said hormone is insulin and the disease treated is non-IDDM type II diabetes.

34. A method according to Claim 31 or 32, wherein said hormone is human growth hormone (HGH) and the diseases treated are disorders in which HGH is involved.

35. A method according to Claim 31 or 32, wherein said hormone is epidermal growth factor (EGF) and the diseases treated are disorders involving EGF.

36. A method for detecting abnormal conditions of a tested cell comprising:

(i) contacting the cells with cyclic glycerophosphates or their analogs (herein CGs) as defined in Claim 13;

(ii) detecting the level of phosphorylation in intracellular proteins of the tested cells; and

(iii) comparing said level of phosphorylation to the level of phosphorylation in intracellular proteins of normal cells following contact with said CGs, a level of phosphorylation differing from that detected in the normal cells indicating a high probability of abnormality in the tested cells.

37. A method according to claim 36 wherein said compound is as defined in claim 1.

38. Use of a compound as defined in Claim 1, for preparation of a medicament for the treatment of disorders and diseases that can be treated by phosphorylation of intracellular proteins.

39. Use of a compound as defined in Claim 13, for the preparation of a medicament for the treatment of malignant diseases and disorders.

40. Use of a compound as defined in Claim 1, for the preparation of a medicament for the treatment of diseases or disorders involving hormone-like signaling.

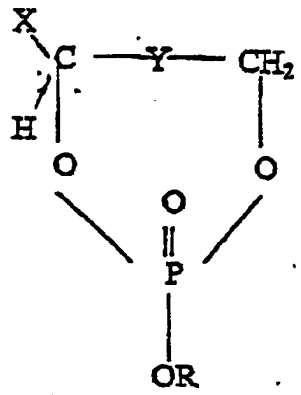
41. Use of a compound according to Claim 22, in the preparation of a medicament.

42. Use of a compound according to Claim 23, in the preparation of a medicament.

43. Use of a compound according to Claim 24, in the preparation of a medicament.

CLAIMS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general formula I:



wherein

Y is $-(CH_2)_m-$, $-CH(OH)-$ or $-C(=O)-$, and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2acyl$; and

R is H, a cation, alkyl or optionally substituted aryl; provided that:

(a) When Y is $-(CH_2)_m-$, m=0, and R is H or cation, X is not CH_2Oacyl; and

(b) Said compound is not one of

(i) Pheny 1,3-cyclic propanediol phosphate,

(ii) Pheny 1,2-cyclic propanediol phosphate,

(iii) Cyclic dihydroxyacetone phosphate,

(iv) 1,3,-cyclic propanediol phosphate

(v) 1,3-cyclic glycerophosphate,

(vi) 1,2-cyclic propanediol phosphate,

(vii) 1,2-cyclic glycerophosphate.

2. A pharmaceutical composition according to Claim 1, wherein said alkyl groups have 1-24 carbon atoms, said acyl groups are aliphatic saturated or

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unsaturated C_1 - C_{24} acyl groups and said aryl group is a carbocyclic aryl group optionally substituted by C_1 - C_4 alkyl, halogen and/or hydroxy.

3. A pharmaceutical composition according to Claim 2, wherein said acyl groups are derived from natural fatty acids.

4. A pharmaceutical composition according to Claim 3, wherein said acyl group is a saturated aliphatic acyl group selected from acetyl, butyryl, caproyl, octanoyl, decanoyl, lauroyl, myristyl, palmitoyl and stearoyl, or an unsaturated aliphatic acyl group selected from palmitoleyl, oleyl, linoleyl, and ricinoleyl.

5. A pharmaceutical composition according to any one of Claims 1-4, wherein said aryl group is phenyl.

6. A pharmaceutical composition according to Claim 1, comprising phenyl 1,2-cyclic glycerophosphate.

7. A pharmaceutical composition according to Claim 1, comprising 3-acyl 1,2-cyclic glycerophosphate.

8. A pharmaceutical composition according to Claim 1, comprising cyclic oleyl lysophosphatidic acid.

9. A pharmaceutical composition according to Claim,1 comprising phenyl 1,3-cyclic glycerophosphate.

10. A pharmaceutical composition according to Claim1, comprising phenyl cyclic dihydroxyacetone phosphate.

11. A pharmaceutical composition for inducing phosphorylation in intracellular proteins of target cells comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of general Formula I of Claim 1.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general Formula I of Claim 1 for promotion of cell differentiation in target cells.

13. A pharmaceutical composition for the treatment of malignant diseases and disorders comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general Formula I of Claim 1 wherein

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Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH2Oacyl or -CH2acyl; and

R is H, a cation, alkyl or optionally substituted aryl; provided that

when Y is $-(CH2)_m-$, m=0, and R is H or cation, X is not CH_2Oacyl.

14. A pharmaceutical composition according to Claim 13, wherein said malignant disorder is a blood malignancy.

15. A pharmaceutical composition according to Claim 14, wherein said blood malignancy is leukemia.

16. A pharmaceutical composition according to Claim 13, wherein said malignancy is breast cancer.

17. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound as defined in Claim 1, for induction of hormone-like signaling.

18. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a as defined in Claim 13, for induction of hormone-like signaling wherein said hormone is selected from the group consisting of insulin, human growth hormone, and epidermal growth factor.

19. A pharmaceutical composition according to Claim 17 or 18 wherein said hormone is insulin and the composition is for the treatment of non-insulin-dependent diabetes mellitus (non-IDDM type II diabetes).

20. A pharmaceutical composition according to claim 17 or 18, wherein said hormone is human growth hormone (HGH) for the treatment of disorders in which HGH is involved.

21. A pharmaceutical composition according to Claim 17 or 18, wherein said hormone is epidermal growth factor (EGF) for the treatment of disorders involving EGF.

22. A compound as defined in claim 1.

23. A compound as defined in Claim 1, with the exception of the following compounds:

(i) compounds wherein Y is - $(CH_2)_m$ -, m is 0, X is CH_3, -CH_2OH or CH_2Oacyl wherein acyl is a saturated carboxylic acyl with more than 12 carbon atoms, and R is H or a cation;

(ii) compounds wherein Y is - $(CH_2)_m$ -, m is 1, X is H and R is H , a cation or phenyl; and

(iii) compounds wherein Y is – CH(OH) - , X is H and R is H, a cation or phenyl.

24. A compound according to Claim 29, selected from the group consisting of:

(i) phenyl 1,2 cyclic glycerophosphate;

(ii) phenyl cyclic dihydroxyacetone phosphate; and

(iii) cyclic oleyl lysophosphatidic acid.

25. A method for treatment of disorders and diseases which can be treated by phosphorylation of intracellular proteins comprising administering to the individual in need a therapeutically effective amount of a compound as defined in Claim 13.

26. A method according to claim 25 wherein said compound is a compound as defined in claim 1.

27. A method for the treatment of malignant diseases comprising administering to an individual in need a therapeutically effective amount of a compound as defined in claim 13.

28. A method according to Claim 27, wherein said malignant disease or disorder is blood malignancy.

29. A method according to Claim 28, wherein said blood malignancy is leukemia.

30. A method according to Claim 27, wherein said malignant disease is breast cancer.

31. A method for the treatment of diseases involving hormone-like signaling comprising administering to an individual in need a therapeutically effective amount of a compound as defined in Claim 13.

32. A method according to claim 31 wherein said compound is as defined in claim 1.

33. A method according to Claim 31 or 32, wherein said hormone is insulin and the disease treated is non-IDDM type II diabetes.

34. A method according to Claim 31 or 32, wherein said hormone is human growth hormone (HGH) and the diseases treated are disorders in which HGH is involved.

35. A method according to Claim 31 or 32, wherein said hormone is epidermal growth factor (EGF) and the diseases treated are disorders involving EGF.

36. A method for detecting abnormal conditions of a tested cell comprising:

(i) contacting the cells with cyclic glycerophosphates or their analogs (herein CGs) as defined in Claim 13;

(ii) detecting the level of phosphorylation in intracellular proteins of the tested cells; and

(iii) comparing said level of phosphorylation to the level of phosphorylation in intracellular proteins of normal cells following contact with said CGs, a level of phosphorylation differing from that detected in the normal cells indicating a high probability of abnormality in the tested cells.

37. A method according to claim 36 wherein said compound is as defined in claim 1.

38. Use of a compound as defined in Claim 1, for preparation of a medicament for the treatment of disorders and diseases that can be treated by phosphorylation of intracellular proteins.

39. Use of a compound as defined in Claim 13, for the preparation of a medicament for the treatment of malignant diseases and disorders.

40. Use of a compound as defined in Claim 1, for the preparation of a medicament for the treatment of diseases or disorders involving hormone-like signaling.

41. Use of a compound according to Claim 22, in the preparation of a medicament.

42. Use of a compound according to Claim 23, in the preparation of a medicament.

43. Use of a compound according to Claim 24, in the preparation of a medicament.

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WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 31/00

A2

(11) International Publication Number: WO 00/57864

(43) International Publication Date: 5 October 2000 (05.10.00)

(21) International Application Number: PCT/IL00/00184

(22) International Filing Date: 24 March 2000 (24.03.00)

(30) Priority Data:
129179 25 March 1999 (25.03.99) IL

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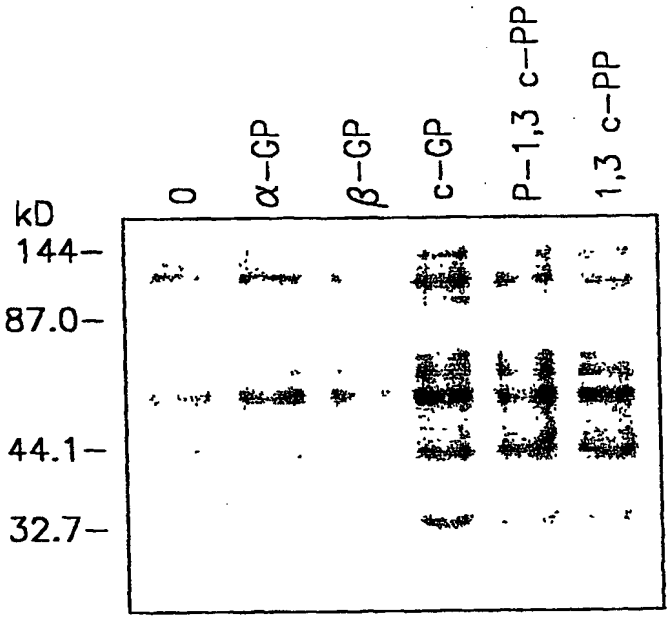
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: CYCLIC GLYCEROPHOSPHATES AND ANALOGS THEREOF

(57) Abstract

Cyclic glycerophosphates as well as some analogs thereof (CGs) are shown to increase phosphorylation of intracellular proteins in various cells. Such activity is not found with linear α or β glycerophosphates. The phospharylating activity of the CGs render them useful in the prevention and treatment of various disorders and diseases such as, for example, different kinds of malignancies as well as disorders involving hormone and hormone–like signaling. The CGs are also useful for promotion of target cell differentiation and for detection of abnormal conditions in target cells.



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CYCLIC GLYCEROPHOSPHATES AND ANALOGS THEREOF

FIELD OF THE INVENTION

The present invention concerns pharmaceutical compositions comprising cyclic glycerophosphates and analogs thereof and some novel compounds of this type.

PRIOR ART

The following is a list of references which is intended for a better understanding of the background of the present invention.

Boyd, R.K., De Freitas, A.S.W., Hoyle, J., McCulloch, A.W., McInnes, A.G., Rogerson, A. and Walter, J.A., *J. Biol. Chem.*, **262**:12406-12408 (1987).

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Su, B., Kappler, F., Szwergold, B.S. and Brown, T.R., *Cancer Res.*, **53**:1751-1754, (1993).

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BACKGROUND OF THE INVENTION

L-α-glycerophosphate (αGP), a key constituent in phospholipid metabolism (Kennedy and Weiss, 1956), is abundant in most biological tissues (Dawson, 1958). β-Glycerophosphate (βGP) is a product of enzymatic (Ukita *et al.*, 1955) and alkaline (Clarke and Dawson, 1976) hydrolysis of phospholipids and is formed through the cyclic phosphodiester intermediate 1,2-cyclic glycerophosphate (1,2 cGP) (Ukita *et al.*, 1955; Clarke and Dawson, 1976). 1,2 cGP has been detected in algae species (Boyd *et al.*, 1987) as well as in human cancer tissues (Su et al., 1993). Similarly, αGP can in principle adopt the cyclic form 1,3-cyclic glycerophosphate (1,3 cGP). This compound has been shown to be formed as an intermediate in the phospholipase C hydrolysis of phosphatidyl glycerol (PG) (Shinitzky *et al.*, 1993) and upon further hydrolysis is converted to αGP.

A six-membered cyclic phosphate of foremost biological importance is cyclic AMP. The ring of cyclic AMP is actually a derivative of 1,3 cGP backbone. Other cyclic phosphates which were detected in biological systems include glucose cyclic phosphodiester (Leloir, 1951), 2',3'-cyclic phosphodiester (Markham and Smith, 1952), riboflavin-4',5'-cyclic phosphodiester (Forrest and Todd, 1950), myoinositol-1,2-cyclic phosphodiester (Dawson *et al.*, 1971) and cyclic lysophosphatidic acid (Friedman *et al.*, 1996).

Except for cyclic AMP and cyclic GMP which have been extensively studied, no specific biological activities have been so far assigned to the other biological cyclic phosphates.

List of compounds and their abbreviations

The following compounds which formulas are presented in **Appendix A** just before the claims, will be represented herein in the specification by their abbreviations as follows:

1. 1,3 cyclic glycerophosphate - **1,3 cGP**
2. 1,2 cyclic glycerophosphate - **1,2 cGP**
3. 3-acyl 1,2 cyclic glycerophosphate (cyclic lysophosphatidic acid) - **c-lysoPA**
4. Phenyl 1,3 cGP – **P-1,3 cGP**
5. Phenyl 1,2 cGP – **P-1,2 cGP**
6. 1,3 cyclic propanediol phosphate – **1,3 cPP**
7. 1,2 cyclic propanediol phosphate – **1,2 cPP**
8. Phenyl 1,3 cPP – **P-1,3 cPP**
9. Phenyl 1,2, cyclic propanediol phosphate – **P-1,2, cPP**
10. Cyclic dihydroxyacetone phosphate – **cDHAP**
11. Phenyl cyclic dihydroxyacetone phosphate - **P-cDHAP**

GLOSSARY

The following is an explanation of some terms used above and in the following description and claims:

CG – the cyclic glycerophosphates and analogs thereof of the invention.

Target cells – cells in which, following contact with the CGs of the invention, there is phosphorylation of intracellular proteins. In some cases, contact of the target cells with CGs results in maturation of the cells and in other cases in hormone-like

signaling activities. In addition, a variety of cellular events may occur in the target cells following their contact with the CGs of the invention.

Intracellular phosphorylation (used interchangeably with phosphorylation of intracellular proteins) – rise in the level of phosphorylation in one or more of the intracellular proteins of the target cells following incubation of the cells with the CGs of the invention. The phosphorylation is typically of the tyrosine amino acid in the protein but may also be of the threonine or serine amino acid. The protein may be any protein inside the target cell that can be phosphorylated. Typically the protein in which phosphorylation occurs is constitutively phosphorylated to some extent and the level of its phosphorylation is effected by the CGs. The level of phosphorylation may be determined using any of the methods known in the art such as those described below.

Promotion of cell differentiation – the activity of the CGs of the invention causing changes in the target cells which are correlated with the differentiation stage of the cells. The changes may be in anatomical characteristics, in the expression of differentiation antigens, etc.

Induction of hormone-like signaling - the activity of the CGs of the invention on target cells which results in changes which are typically induced by hormones. The CGs applied externally to the target cells pass through the cell membrane and exert their effect inside the target cells. For example, in target cells expressing the insulin receptor, such changes may be similar to the effects exerted by insulin on the same cells.

Analog - relates to any compound which is derived from one of the cyclic glycerophosphates of the invention and which substantially maintains the activity of the cyclic glycerophosphate from which it was derived, including, for example,

deoxy analogs and phenyl esters of the cyclic glycerophosphates, preferably, deoxy analogs.

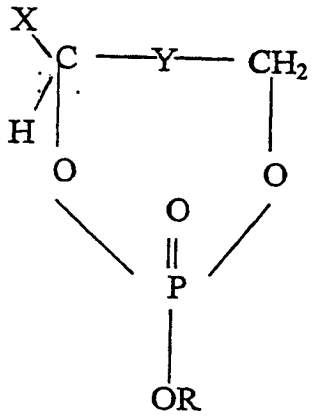
Substantially maintaining - this term relates to the analogs ability to promote the activity carried out by the cyclic glycerophosphate from which they were derived to a certain extent. The analog's activity will be considered to be substantially maintained wherein the activity is 30% or above, preferably 50% or above, more preferred 70% or above, and most preferably 90% or above the level of the activity of the cyclic glycerophosphate.

Effective amount – wherein the method of the invention is intended for prevention of a non-desired condition, the term *"effective amount"* should then be understood as meaning an amount of the active compound which, when administered, results in the prevention of the appearance of the said condition. Prevention of such a condition may be required prior to the appearance of any symptoms of a disease, e.g. in individuals having a high disposition of developing the disease. Wherein the compositions or methods are intended for treatment of an ongoing non-desired condition, the term *"therapeutically effective amount"* should then be understood as meaning an amount of the active compound which is effective in ameliorating or preventing the enhancement of the treated condition and related symptoms, which reduces the undesired symptoms or which completely eliminates them.

SUMMARY OF THE INVENTION

It has now been found, in accordance with the present invention, that extracellular application of cyclic glycerophosphates and analogs thereof to target cells increases within minutes the level of phosphorylation in intracellular proteins of the cells. Linear αGP and linear βGP on the other hand, lack this activity.

The present invention thus provides, by a first of its aspects, a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general formula I:



(I)

wherein

Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl.

As used herein the term *"alkyl"* refers to an alkyl group having from about 1 to about 24 carbon atoms, e.g. preferably from about 3 carbon atoms to about 20 carbon atoms, most preferably from about 5 carbon atoms to about 15 carbon atoms; the term *"acyl"* refers to an aliphatic saturated or unsaturated C_1 - C_{24} acyl group, preferably an acyl group having an even number of carbon atoms, most preferably an acyl group derived from a natural fatty acid such as a saturated aliphatic acyl group selected from acetyl, butyryl, caproyl, octanoyl, decanoyl, lauroyl, myristyl, palmitoyl and stearoyl, or an unsaturated aliphatic acyl group selected from palmitoleyl, oleyl, linoleyl, and ricinoleyl; and the term *"aryl"* refers to a mono- or poly-carbocyclic aryl group, most preferably phenyl, optionally substituted by C_1 - C_4 alkyl, halogen and/or hydroxy. R may be any physiologically suitable cation and is preferably Na^+.

In one embodiment, Y is –CH(OH)-, X is H and R is H or phenyl. According to this embodiment, the composition comprises 1,3 cyclic glycerophosphate (1,3 cGP) or phenyl 1,3 cyclic glycerophosphate (P-1,3 cGP).

In another embodiment, Y is –C(=O)-, X is H and R is H or phenyl. According to this embodiment, the composition comprises cyclic dihydroxyacetone phosphate (cDHAP) or phenyl cyclic dihydroxyacetone phosphate (P-cDHAP).

In a further embodiment, Y is $-(CH_2)_m-$, m is 0, X is $-CH_2OH$ and R is H or phenyl. According to this embodiment, the composition comprises 1,2 cyclic glycerophosphate (1,2 cGP) or phenyl 1,2 cyclic glycerophosphate (P-1,2 cGP).

In still a further embodiment, Y is $-(CH_2)_m-$, m is 0, X is a $C_1 - C_{24}$ alkyl, preferably $-CH_3$, and R is a cation or phenyl. According to this embodiment, the composition comprises 1,2 cyclic propanediol phosphate (1,2 cPP) or phenyl 1,2 cyclic propanediol phosphate (P-1,2 cPP).

In yet still a further embodiment, Y is $-(CH_2)_m-$, m is 1, X is a $C_1 - C_{24}$ alkyl, preferably $-CH_3$, and R is a cation or phenyl. According to this embodiment, the composition comprises 1,3 cyclic propanediol phosphate (1,3 cPP) or phenyl 1,3 cyclic propanediol phosphate (P-1,3 cPP).

In yet another embodiment, Y is $-(CH_2)_m-$, m is 0, X is $-CH_2$ (C_1-C_{24})acyl, preferably oleyl, and R is a cation. According to this embodiment, the composition comprises 3-acyl- 1,2 cyclic glycerophosphate (cyclic lisophosphatidic acid – c-lyso PA).

In another aspect, the invention relates to novel compounds of the above Formula I with the exception of the following compounds: (i) compounds wherein Y is $-(CH_2)_m-$, m is 0, X is CH_3, $-CH_2OH$ or CH_2Oacyl wherein acyl is a saturated carboxylic acyl with more than 12 carbon atoms, and R is H or a cation; (ii) compounds wherein Y is $-(CH_2)_m-$, m is 1, X is H and R is H, a cation or phenyl; and (iii) compounds wherein Y is -CH(OH)-, X is H and R is H, a cation or phenyl.

Examples of the new compounds are the compounds:

phenyl 1,2 cyclic glycerophosphate

phenyl 1,2 cyclic propanediol phosphate

cyclic dihydroxyacetone phosphate

phenyl cyclic dihydroxyacetone phosphate

cyclic oleyl lysophosphatidic acid.

By another of its aspects, the present invention provides a pharmaceutical composition for inducing phosphorylation in intracellular proteins of target cells comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of general Formula I above.

Such phosphorylation of proteins is known to be an essential stage of many signaling pathways which are involved in cellular processes. The phosphorylating activity of the cyclic glycerophosphates and analogs thereof of the invention renders them useful in the prevention and treatment of various disorders and diseases.

The present invention also provides a pharmaceutical composition for treatment of disorders and diseases which can be treated by phosphorylation of intracellular proteins comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of general Formula I above.

In addition, the present invention provides a method for treatment of disorders and diseases which can be treated by phosphorylation of intracellular proteins comprising administering to the individual in need a therapeutically effective amount of a compound of general Formula I above.

One cellular process which involves phosphorylation in intracellular proteins is cell differentiation. The present invention also provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active material, a compound of the general Formula I above for promotion of cell differentiation in target cells.

The capability of the compositions of the invention to induce cell differentiation in target cells makes them especially suitable for use in the treatment of various disorders and diseases such as various malignancies. In accordance with the invention it was shown, for example, that the cyclic phosphate 1,3 cGP increases the expression of estrogen receptor on breast cancer

tumor cells in culture. This makes these compounds good candidates for treatment of breast cancer as well as blood malignancies such as leukemias and lymphomas and other solid tumors such as brain tumors, etc.

By an additional aspect, the present invention also provides a method for the treatment of malignant diseases comprising administering to an individual in need a therapeutically effective amount of the compound of Formula I above.

In addition, due to the capability of the compositions of the invention to induce hormone-like signaling, they may be used for the prevention or treatment of various disorders in which such hormone signaling is involved.

By yet another of its aspects, the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active material, a compound of the general Formula I above for induction of hormone-like signaling.

The hormone-like signaling activity of the pharmaceutical composition of the invention may affect the target cell in a similar manner as that of the typical hormone which affects the cells and/or may be synergistic with the activity of the hormone resulting in an elevated signal in the treated cells. In accordance with this aspect of the invention, the CGs may, for example, be used for induction of an insulin-like signal. This may be useful in the treatment of non-insulin dependent diabetes mellitus (non-IDDM) – type II diabetes. In addition the CGs may be used for induction of the signal of human growth hormone (HGH) in the treatment of disorders in which HGH is involved, for induction of epidermal growth factor (EGF) for use in the treatment of disorders involving EGF, etc.

By yet another of its aspects, the present invention provides a method for the treatment of diseases involving hormone-like signaling comprising administering to an individual in need a therapeutically effective amount of the compound of the Formula I above.

In addition, the compounds of the invention may be used to prepare medicaments suitable for treating diseases and disorders such as those mentioned above.

Being involved in crucial signaling pathway of the cell, the level of phosphorylation of intracellular proteins may be used as an indicator of certain cellular situations. For example, there may be disorders which affect the activity of kinase enzymes or phosphatase enzymes resulting in abnormal levels of phosphorylation in response to extracellular stimulations. By contacting cells of interest with the CGs of the invention, it is possible to measure the level of phosphorylation in the cells and to compare it to the level of phosphorylation in normal cells. A level of phosphorylation which differs from that in normal cells may indicate an abnormal condition in the tested cells. Thus, the present invention also provides a method for detecting abnormal conditions of a tested cell comprising:

i. contacting the cells with a CG of the invention;

ii. detecting the level of phosphorylation in intracellular proteins of the tested cells; and

iii. comparing said level of phosphorylation to the level of phosphorylation in intracellular proteins of normal cells following their contact with said CGs, a level of phosphorylation differing from that detected in the normal cells indicating a high probability of abnormality in the tested cells.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows the level of tyrosine phosphorylated proteins in CHO cells after 1 min. pulsing at 37°C with 1μM αGP, βGP, 1,3 cGP (E), P-1,3 cPP and 1,3 cPP. Detection with polyclonal anti-phosphotyrosine antibodies.

Fig. 2 shows the level of tyrosine phosphorylated proteins in CHO cells after 1 min pulsing at 37°C with 1μM and 2μM 1,3 cPP. Detection with monoclonal anti-phosphotyrosine antibodies.

Fig. 3 shows the level of tyrosine phosphorylated proteins in NIH 3T3 cells after 1 min pulsing at 37°C with 1,3 cPP. Detection with monoclonal anti-phosphotyrosine antibodies.

Fig. 4 shows the time course of tyrosine phosphorylation in proteins of CHO cells pulsed with 5μM and 10μM 1,2 cPP for a period of 1 min., 3 mins. or 5 mins. . Detection with monoclonal anti-phophotyrosine antibodies.

Fig. 5 shows the ^{32}P labelled proteins of CHO cells after 2 min pulsing with 2μM 1,3 cGP (E) or 1,3 cPP followed by gel electrophoresis and in-gel treatment with 1 M KOH.

Fig. 6 shows the in-gel assay for kinase identification of proteins from CHO cells phosphorylated by 2μM 1,3 GP or 1,3 cPP in comparison to 2 μM αGP or βGP (control). In-gel identification of Raf-1, MAPK and Jun kinase in the phosphorylated proteins is shown in the lower panel.

Fig. 7 shows the time profile of threonine phosphorylation in CHO cells induced by 4μM 1,3 cGP (E) at 25°C. Abolishment of detection of threonine phosphorylation (a) by the presence 5 mM phosphoserine (b) or 5 mM phosphothreonine (c) is also shown.

Fig. 8 shows the uptake profile of 1,3 $cP^{32}P$ (10μM) by CHO cells. The inserts presents the magnified profile for short incubation times.

Fig. 9 shows the displacement profiles of 1,3 $cP^{32}P$ in perforated CHO cells by αGP (x-x), unlabelled 1,3 cPP (filled symbols, 3 separate experiments) and 1,3 cGP (E) (open symbols, 2 separate experiments).

Fig. 10 is a schematic representation showing the relative level of phosphorylation of insulin receptors on CHO-T cells following their incubation with insulin (1 nM or 0.1 nM), 1,3, cPP (0.1 μM) or a combination of both. **Fig. 10A** shows phosphorylation of the 117 kD band of the insulin receptor and **Fig. 10B** shows phosphorylation of the 200 kD band of the insulin receptor.

Fig. 11 is a schematic representation showing the level of expression of the estrogen receptor (ER) in human ER^{mod} $T_{47}D$ cells incubated with the cyclic phosphate 1,3 cGP or in the culture medium as control. The results are shown 1, 4 and 7 days after beginning of incubation.

Fig. 12 is a schematic representation showing the level of proliferation of $T_{47}D$ human breast cancer cells grown *in vitro* either with growth medium or with a final concentration of 50 μM of 1,3-cPP salt. The effect on proliferation is shown on days 1, 2 and 3 of the culture.

Fig. 13 is a schematic representation showing the amount of hemoglobin production in K562 leukemia cells following their incubation for four days with growth medium (control), 1,3 cPP, α-GP, a differentiation-inducing agent sodium butyrate and combinations thereof.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention it has been found that cyclic glycerophosphates and some of their analogs (all herein designated *"CGs"*), are involved in signaling pathways in cells. Linear α and β glycerophosphates do not exert such an activity.

Cyclic glycerophosphates can be formed by enzymatic degradation of phospholipids which in most cases yields five or six membered ring cyclic phosphates. The present invention encompasses within its scope both such cyclic glycerophosphates formed by enzymatic degradation of phospholipids as well as synthetically formed ones. CGs having rings of less than five or more than six carbon atoms are also included within its scope.

The cyclic glycerophosphates and analogs of the invention may generally be synthesized using any one of the methods known in the art for synthesis of phosphate esters. Specific methods which may typically be used for preparing the cyclic phosphates of the invention are described specifically below (see Examples).

Analogs of these cyclic glycerophosphates of the invention are also within the scope of the invention being typically deoxy analogs as well as phenyl esters of the 1,3 cyclic phosphates. These analogs may also be prepared by enzymatic methods or synthetically by any of the methods known in the art.

In addition to the active ingredient, the pharmaceutical compositions of the invention may also contain a carrier selected from any one of the carriers known in the art. The nature of the carrier will depend on the intended form of administration and indication for which the composition is used. The compositions may also comprise a number of additional ingredients such as diluents, lubricants, binders, preservatives, etc.

The signaling activity of the CGs of the invention is exerted through their capability to induce phosphorylation of intracellular proteins in cells. When the CGs are applied onto the cells, a rapid phosphorylation is observed in intracellular proteins. Typically, the phosphorylating activity of the CGs may be observed after a period of between about 0.5 mins. to about 20 mins. of contact with the target cells.

In accordance with the invention, the phosphorylating activity of the CGs may be measured by any of the methods known in the art. Generally, following incubation of the CGwith the target cells, the cells are lysed, the protein concentration in each sample is determined and the level of phosphorylation of the proteins is determined using known methods and suitable and available polyclonal or monoclonal antibodies. Typically, phosphorylation occurs on the tyrosine residues of the protein and such phosphorylation is determined using either polyclonal or monoclonal anti phosphotyrosine antibodies (such as those described below in the Examples). However, in some cases, phosphorylation occurs on the threonine or serine components of the proteins in which case polyclonal or monoclonal anti phosphothreonine or anti phosphoserine antibodies may be used.

The molecular weight of the proteins in which phosphorylation occurs following incubation with various kinds of CGs may also vary to a certain extent. Thus, for example, incubation of CHO and NIH-3T3 cells with six membered ring cyclic phosphates resulted in phosphorylation of proteins having a molecular weight of about 35 kD, about 45 kD, about 60 – 70 kD and about 120 kD. The molecular weight of the proteins found to be phosphorylated following incubation of the same cells with five membered ring cyclic phosphates was about 18 kD, about 35 kD and about 38 kD.

The CGs of the invention may be administered to cells *in vitro*. Such administration may result in desired changes in the cells which may then be administered back to an individual in need. In addition, administration of the compositions to cells may result in enhanced secretion of various growth factors by these cells which, in turn, could also be used for treatment of various conditions alone or in combination with other CGs of the invention.

The effective amount of the CG to be used *in vitro* in accordance with the invention may vary in accordance with the nature of the CG as well as the target cells and can easily be determined by a person versed in the art by using any of the above-mentioned methods or any of the methods known in the art. For *in vitro* induction, the typical range of concentration of CG needed to induce phosphorylation is between about 0.5 μM to about 10 μM.

Many cellular processes are triggered by protein phosphorylation (mostly tyrosine phosphorylation). Thus, application of the CGs to target cells results in various cellular processes. One such process, is cell differentiation. Such differentiation may easily be determined by a person skilled in the art by measurement of parameters and characteristics in the target cells upon their differentiation. The CGs capability of inducing differentiation of cells makes them useful in the prevention or treatment of disorders or diseases in which differentiation of cells is desired such as, for example, in various malignant diseases.

The CGs of the invention are also able to exert hormone-like signaling activities in target cells. Thus, for example, the CGs of the invention may exert insulin-like activity on cells expressing the insulin receptor. This makes the CGs of the invention suitable candidates for treatment of disorders or diseases involving hormone signaling. The CGs of the invention may also be used in synergism with known hormones, e.g. together with insulin in the treatment of IDDM.

Where the compositions of the invention are administered *in vivo*, a preferred mode of their administration is either i.v., topically or per os although at times it may be advantageous to use other administration modes as well.

Typically, the pharmaceutical compositions of the invention will comprise about 1 mg to about 10 mg of the active material per kg body weight of the treated individual.

While the compositions of the invention will typically contain a single CG, it is possible at times to include in the composition or to co-administer two or more

CGs which may then act together in a synergistic or additive manner to prevent or treat the specific disorder.

According to the invention, the CGs may be administered either in a single dose or may be given repetitively over a period of time.

The compositions of the invention may also be administered to the treated individual in combination with an additional treatment, e.g. wherein the treated condition is a malignant one, the compositions may be given together with one of the currently available drugs or therapies used for treatment of such diseases such as various chemotherapeutic drugs together with a growth factor such as Interleukin-2 (IL-2). In another example, the CGs of the invention may be administered to an individual suffering from IDDM in combination with insulin. In such a combination treatment the CGs may be administered simultaneously with or at different times than the administration of the additional treatment so as to yield a maximum preventive or therapeutic effect.

The induced increase in intracellular phosphorylation by cyclic phosphates of the invention may be the result of the effect of the cyclic phosphates on one of several routes including activation of intracellular kinases (e.g. MAPK) on the one hand and inhibition of phosphatase activity on the other hand. Each of the above routes which may occur separately or in combination results in the augmentation of the degree of phosphorylation of intracellular proteins. The apparent degree of phosphorylation of such proteins is, most likely, at a steady state between counteracting kinase and phosphatase activities.

Without being bound by theory, and on the basis of the results of the present invention, the CGs of the invention may exert their activity in the following way: upon application of the cyclic phosphate to the target cell, the CG first partitions into the cytosol of the cell and when it reaches certain local concentration (of between about 0.1 to about 1 μM) is capable of activating kinases in the cytosol (such as MAPK). At the same time the CGs also inhibit phosphatase activity. The activation of kinases and inhibition of phosphatase activity results in induction of phosphorylation of tyrosine or serine/threonine phosphorylations in a series of

proteins. As the concentration of the CGs in the cytosol rises, phosphodiester activity cleaves the active cyclic phosphates reducing their above activities and resulting in a reduction in the phosphorylation level of proteins in the cells.

The cyclic phosphates of the invention may be used in any of their isomer forms. For various purposes, one of the isomers may be preferred over the remaining ones. For example, amongst the four stereo isomers which constitute the synthetic 1,3 cGP depicted in Appendix A, the enzymatic product 1,3 cGP(E) is preferred for use for inhibiting the overall intracellular phosphatase activity.

EXAMPLES

The invention will now be illustrated by the following non-limiting examples with reference to the appended figures.

CHEMICAL SECTION

Synthesis of the cyclic phosphates

The cyclic phosphates of the invention are prepared by the reaction of a suitable dihydroxy compound wherein Y is - $(CH_2)_m$ - or - C(=O) - and X is H or alkyl with phosphorus oxycloride ($POCl_3$) when R is H or with aryl, e.g. phenyl, phosphorodichloridate (RO-P(=O)Cl_2) when R is aryl.

When there is one or more hydroxy groups in the starting compound, namely Y is - CH(OH) - and/or X is - CH_2OH -, these hydroxy groups have to be protected, e.g. by benzylation, and the benzyl group is then removed after cyclization by conventional catalytic hydrogenation in the presence of a suitable catalyst such as Pt or Pd.

The reaction is carried out in an anhydrous solvent, e.g. dioxane or methylene chloride, in the presence of equivalent amounts of a nucleophile such as pyridine or triethylamine. The end products, when R is not aryl, are usually obtained as salts.The synthesis of a series of known and novel 5- and 6-membered ring cyclic phosphates is illustrated below.

Example 1: Synthesis of 1,3 cyclic glycerophosphate (1,3 cGP) and 1,3,cGP(E)

The procedure of Buchnea (Buchnea, 1973) was followed essentially as described. Briefly, 2-benzyloxy-1,3-propanediol (Aldrich) was reacted with an equimolar amount of phosphorus oxychloride (Aldrich) in methylene chloride. The resulting 2-benzyl-1,3 cGP was treated with hydrogen under the catalysis of Pd black in methanol to remove the benzyl residue. The 1,3 cGP, isolated as the Ba salt, was pure on paper chromatography (n-propanol: ammonia: water 6:3:1, R_f=0.52).

1,3 cGP was also produced by the cleavage of phosphatidyl glycerol (PG) with phospholipase C as described (Shinitzky et al., 1993). The product, termed 1,3,cGP(E) had a trace of approx. 10-20% α-GP as indicated by paper chromatography.

Example 2: Synthesis of 1,2 cyclic glycerophosphate (1,2 cGP)

This compound was prepared as described (Kugel and Halmann, 1967). The disodium salt of β-glycerophosphate (Sigma) was first converted to the acid form and then cyclized with dicyclohexylcarbodiimide (Aldrich). The product, isolated as the Ba salt, was pure on paper chromatography.

Example 3: Synthesis of phenyl 1,3 cyclic glycerophosphate (P-1,3 cGP)

The method described in Example 1 for 1,3 cGP was followed by reacting 2-benzyloxy-1,3-propanediol with phenyl phosphorodichloridate (Aldrich). The intermediate benzylated product was pure on thin layer chromatography (ethyl acetate:hexane 3:2 R_f =0.58), with a melting point of 136°C. It was further hydrogenated as in Example 1 to remove selectively the benzyl residue. The obtained P-1,3 cGP, compound **III**, was pure on thin layer chromatography (as above) with R_f=0.15 and melting point of 116°C.

Example 4: Synthesis of 1,3 cyclic propanediol phosphate (1,3 cPP)

1,3 cPP was prepared by reacting 1,3-propanediol (Aldrich) with an equimolar amount of phosphorus oxychloride and then purified as described by Buwalda et al., 1997. The product was isolated as the free acid (melting point: 99-100°C).

^{32}P labeled 1,3 cPP (1,3 $cP^{32}P$) was prepared with $^{32}POCl_3$. The latter was obtained by introducing a trace of $H_3{}^{32}PO_4$ (Amersham) into an excess of $POCl_3$ in the cold (Neuhaus and Korkes, 1958). The reaction was then proceeded on a microscale and 1,3 $cP^{32}P$ was isolated by co-crystallization with unlabelled 1,3 cPP.

Example 5: Synthesis of 1,2 cyclic propanediol phosphate (1,2 cPP)

1,2 cPP was prepared by the same procedure as in Example 4 but using 1,2- propanediol (Aldrich). The compound was isolated as the Ba salt and was pure on paper chromatography (n-propanol:ammonia:water 6:3:1, R_f=0.55).

Example 6: Synthesis of phenyl 1,3 cyclic propanediol phosphate (P-1,3 cPP)

P-1,3 cPP was prepared by a method analogous to the procedure of Example 4, by reaction of 1,3-propanediol with an equimolar amount of phenyl phosphorodichloridate in dry pyridine. The product was crystallized twice from ethyl acetate-hexane and had a melting point of 72°C.

Example 7: Synthesis of phenyl 1,2 cyclic glycerophosphate (P-1,2 cGP)

This novel compound was prepared as in Example 3 by reaction of 1-benzyloxy-2,3-propanediol with phenyl-PO_2Cl_2, followed by removal of the benzyl residue by selective hydrogenation.Crystallization was achieved from ethanol-acetone and the product had a melting point of 95°C.

Example 8: Synthesis of phenyl 1,2 cyclic propanediol phosphate (P-1,2 cPP)

This novel compound was prepared as in Example 6 by reaction of 1,2-propanediol with an equimolar amount of phenyl-PO_2Cl_2 in dry pyridine. Crystallization was achieved from ethyll acetate-hexane and the product had a melting point of 69°C.

Example 9: Synthesis of cyclic dihydroxyacetone phosphate (cDHAP)

This novel compound was prepared by reaction of $POCl_3$ with dihydroxyacetone.

1.8 g (0.01M dimer or 0.02M monomer) Dihydroxyacetone dimer MW-180 dissolved in 20 ml fresh distilled methylene chloride.

3.07 g = 1.87 ml (0.02M) Phosphoryl chloride (MW-153.5, d-1.645) in 4 ml $MeCl_2$ was slowly added to the solution at RT. The solution was refluxed for 15 h (the solution was black). Methylene chloride was evaporated and 100 ml 90% acetone/water was added to the solution. The reaction mixture was refluxed for 18 h. The black solution was treated with active carbon at RT and filtered. From the resulting slightly yellow solution was evaporated acetone and water and the very nice crystalline residue was dissolved in 10 ml acetone. 0.01 M BaJ_2 in 80 ml acetone was added to the solution and nice crystals of cyclic-dihydroxyacetone-phosphate barium salt started to precipitate. The precipitate was washed 3 times with small quantities of acetone and dried. The product was cleaned by dissolving it in small amounts of water and precipitating with acetone. The resulting produce is white crystalline powder and shows in paper chromatography (solvents mixture: n-Propanol:NH_4H_2O 6:3:1) R_f – 0.50.

Example 10: Synthesis of phenyl cyclic dihydroxyacetone phosphate (P-cDHAP)

This novel compound was prepared by reaction of phenyl-PO_2Cl_2 with dihydroxyacetone in dry pyridine. Upon removal of the solvent by vacuum, the

residue was extracted twice with ethyl acetate. After evaporation of the ethyl acetate, an oily residue was obtained..

Example 11: Synthesis of cyclic oleyl lysophosphatidic acids (c-lysoPA)

These novel compounds were prepared by reaction of oleyl lysophosphatidic acid (Avanti Polar Lipids) with excess dicyclohexylcarbodiimide (DCC) in dimethyl sulfoxide. The product appeared as a oil.

BIOLOGICAL SECTION

Materials and Methods

(i) Cells

Chinese hamster ovary (CHO) cells (Puck, 1985; Gottesman, 1987) and NIH-3T3 cells (NIH), were used in the following experiments. CHO cells were grown at 37°C in a humidified 5% CO2 atmosphere in 60x15 mm Petri dishes (Falcon) containing F12 medium supplemented with 10% fetal calf serum (FCS) and 2mM glutamine. When reaching near confluence, the cells were placed under overnight starvation by replacing the medium to F12+2 mM glutamine with 0.1% FCS. The stimulation experiments were performed with cells which were under starvation for 12-18 hours.

NIH-3T3 cells were grown under similar conditions in 1640 Eagle medium at 10% bovine calf serum plus 2mM glutamine.

(ii) Tyrosine phosphorylation

The cyclic phosphates 1,3 cGP, 1,3 cPP, 1,2 cGP, 1,2 cPP, P-1,3 cGP and P-1,3 cPP and the controls αGP and βGP were dissolved in HBSS, or methanol to form stocks of 1mM or 10 mM. They were diluted into the cell culture medium to a final concentration of up to 20μM. At the assigned stimulation time (0.5-20 min.) the medium was aspirated under vacuum and the cells were washed 3 times with cold (4°C) phosphate buffered saline (PBS). The cells were then

freeze-thawed 3 times with liquid nitrogen in 0.5ml of a lysis buffer of PBS containing 1mM phenylmethylsulfonyl fluoride, a cocktail of protease inhibitors (aprotinin, leuproptin, pepstatin A, phenanthroline, benzamidine HCl each at a concentration of 10μg/ml) and a cocktail of phosphatase inhibitors (80 mM β-glycerophosphate, 2 mM EGTA and 50 μM Na_3VO_4), and then scraped off. The lysate was then vortexed and centrifuged for 2 minutes at 12.000 g. The supernatant was collected for further determinations by gel electrophoresis. The protein concentration in each sample, as evaluated by the Bradford assay, was in the range of 1-1.5 mg/ml. Aliquots of 10μg protein were applied onto 10% SDS-PAGE in a minigel set and resolved within 1 hour. The proteins were then transferred to nitrocellulose sheets for Western blotting. After blocking with a solution of 1% bovine serum albumine and 0.1% Tween 20 in PBS, the blots were incubated at 4°C for 16 hours with polyclonal rabbit anti-phosphotyrosine antibodies (Zymed Laboratories, San Francisco, CA) or monoclonal anti-phosphotyrosine antibodies (Transduction Laboratories, Lexington KY) and then washed several times. Bound antibodies were detected by horseradish peroxidase-conjugated anti-rabbit antibodies (Transduction Laboratories, Lexington KY) after 2 hours incubation at room temperature, using the conventional ECL detection method. Control assays were carried out in the presence of 5mM phosphotyrosine or without the phosphotyrosine antibodies. In both, the intensity of the bands was reduced to background level.

(iii) Threonine phosphorylation

Blots were tested analogously for threonine phosphorylation using polyclonal rabbit anti-phosphothreonine antibodies (Zymed Laboratories, San Francisco, CA).

(iv) In-gel kinase assay

Phosphorylated cell proteins were separated by SDS-PAGE using a gel that was copolymerized with MBP (0.5mg/ml) and treated as described

(Karunuguran *et al., EMBO J.* **15**:254-264, 1996). After electrophoresis, the gel was fixed with isopropanol and denatured using 6M Urea. Renaturation was achieved by gradual removal of the excess of urea followed by extensive washings in a renaturing buffer (16 h, 4°C) and in a buffer containing 20mM HEPES pH 7.6, and 20 mM $MgCl_2$ (30 min, 30°C). The gel was then subjected to phosphorylation in a buffer containing 20mM HEPES pH 7.6, 20 mM $MgCl_2$, 2mM DTT, 20 μM ATP and 100 mCi [γ^{32}P]-ATP (30°C for 120 min). Finally the gel was extensively washed, dried, and subjected to autoradiography.

(v) Immunoprecipitation kinase assays. Raf-1 activity was determined by immunoprecipitation with anti-Raf-1 C terminus antibodies (Santa Cruz Biotech, CA) using recombinant MEK1 as a substrate as previously described (Seger, 1994). Mitogen-activated protein kinase (MAPK) activity was determined by immunoprecipitation with anti-ERK2 C terminus antibodies (Santa Cruz Biotech, CA) using MBP as a substrate (Seger, 1994). Jun N-terminal kinase (JNK) activity was determined by purifying the JNK on a GST-Jun (1-97) column followed by phosphorylation (Hibi, 1993).

RESULTS

Example 12 Tyrosine phosphorylation in CHO cells by 6-membered ring cyclic phosphates detected with polyclonal antibodies

CHO cells were contacted for 1 min. at 37°C with 1 μM of αGP, βGP, 1,3cGP(E), P-1,3-cPP and the level of tyrosine phosphorylated proteins in the cells was then determined by using polyclonal anti phosphotyrosine antibodies as explained above.

As seen in Fig. 1, augmented tyrosine phosphorylation was induced by all of the above cyclic phosphates in a series of proteins as seen in several major bands having μM of about 35 kD, 45 kD, 60 - 70 kD and about 120 kD.

Example 13 Tyrosine phosphorylation in CHO cells by 6-membered ring cyclic phosphates detected with monoclonal antibodies

CHO cells were contacted for 1 min. with the same linear and cyclo glycerophosphates described in Example 12 above under the same conditions. Determination of the level of tyrosine phosphorylated proteins in the cells was determined using a monoclonal anti phosphotyrosine antibody of the kit described above. Using this antibody, the detected tyrosine phosphorylation was in bands having a molecular weight of about 35 kD and of about 45 kD (results not shown).

Example 14 Tyrosine phosphorylation in CHO cells by 1,3,-cPP at various concentrations

CHO cells were incubated for a period of 1 min., 3 mins., 5 mins. or 10 mins. at 37°C with 1 μM or 2 μM of 1,3-cPP. The level of tyrosine phosphorylated proteins in the cells was determined using monoclonal anti phosphotyrosine antibodies.

As seen in Fig. 2, phosphorylation was most markedly seen in the band(s) having a molecular weight of about 35 kD and about 45 kD.

Example 15 Tyrosine phosphorylation in NIH 3T3 cells by 6-membered ring cyclic phosphates as detected by polyclonal antibodies

The level of tyrosine phosphorylated proteins in NIH 3T3 cells was determined after their incubation for 1 min. at 37°C with αGP, βGP, 1,3cGP(E), P-1-3 cPP. The level of tyrosine phosphorylated proteins in the cells was determined using polyclonal anti phosphotyrosine antibodies.

Augmented tyrosine phosphorylation was induced in the cells by all the above cyclic phosphates (results not shown).

Example 16 Tyrosine phosphorylation in NIH 3T3 cells by 6-membered ring cyclic phosphates as detected by monoclonal antibodies

The level of tyrosine phosphorylated proteins in NIH 3T3 cells was determined as described above after their incubation for either 1 min. or 5 mins. at 37°C with 0.5 μM, 1 μM, 2 μM or 4 μM of 1,3-cPP. The level of tyrosine phosphorylated proteins was determined using monoclonal anti phosphotyrosine antibodies as described above.

As seen in Fig. 3, augmented tyrosine phosphorylation was seen in the cells similar to that seen in the cells of Example 14 above.

Example 17 Tyrosine phosphorylation in NIH 3T3 cells by 5-membered ring cyclic phosphates

Induction of tyrosine phosphorylation in cells by 5-membered ring cyclic phosphate (see Appendix 1) was carried out as described in Example 12 above.

The pattern of tyrosine phosphorylation was similar to that obtained by incubation of the cells with the 6-membered ring cyclic phosphates but at a relatively higher concentration and at longer incubation times (results not shown).

Example 18 Kinetics of tyrosine phosphorylation in CHO cells

The kinetics of tyrosine phosphorylation in proteins of CHO cells incubated with either 5 μM or 10 μM of 1,2-cPP at 37°C for a period of 1 min., 3 mins. or 5 mins. was determined as described above using monoclonal anti phosphotyrosine antibodies. As seen in Fig. 4, enhanced tyrosine phosphorylation was detected at bands having μM of about 35 kD, 60 – 70 kD, 120 kD and an additional band of about 38 kD. Similar results were obtained using the deoxy analog of 1,2-cPP (results not shown).

Example 19 Induced tyrosine phosphorylation in cells as detected by *in situ* ^{32}P labeling

To further verify the induced tyrosine phosphorylation shown in the above examples, *in situ* ^{32}P labeling of CHO cells was carried out under a pulse of cyclic phosphate as described above. 10^7CHO cells/per 1 ml medium were pulsed with 1 mCi of $^{32}PO_4^{-3}$ for 12 hours and then activated for 2 mins. with 2 μM of 1,3-cGP(E) or 2 μM of 1,3-cPP. As control, the same cells following the 12 hour pulsing were not contacted with the cyclic cGPs. Massive ^{32}P protein labeling was observed in both the cells which were incubated with the cyclic GMPs as well as with one set that were not.

However, as seen in Fig. 5, following treatment of the gel with 1M of KOH for 2½ hours at 25°C or 37°C (which selectively hydrolyzes the phosphoserine and phosphothreonine residues (Kozma *et al., J. Methods Enz.*, **201**:28-43, 1991), two major ^{32}P bands of phosphorylated tyrosine at molecular weights of about 35 kD and 45 kD emerged in the treated samples. These bands could be correlated with kinase activity (see Example 20 below).

Example 20 Detection of phosphorylated kinases by in-gel kinase assay

The in-gel kinase assay in the presence of MBP was applied as described in Materials and Methods above to detect stimulation of protein kinases by the cyclic phosphates. CHO cells were treated either with medium (0 control), or with 2 μM, α GP or β GP for 2 minutes, 2 μM 1,3-cGP (E) for 2 and 5 minutes and 2 M 1,3-cPP for 2 minutes after which the cells were lysed and analyzed.

As seen in Fig. 6, at least three MBP phosphorylated kinases could be detected by this method . Their apparent molecular weights were around MW of 35-40, 55 and 62 kD. The 35-40 kD protein kinase which was activated by 1,3-cGP (E) could be assigned to the P42 MAPK (ERK 2). In addition, cells were treated as described above and then subjected to Raf-1, MAPK and IP kinase assays and these are presented in the first and second rows of Fig. 6. In parallel,

JNK assay was performed (third row, JST-Jun substrate). The results strongly suggest that the Raf1/MAPK cascade is activated up to approximately 5 fold by 1,3-cGP (E), whereas the JNK pathway seems to be unaffected.

Example 21 Augmentation of threonine phosphorylation by 6-membered ring cyclic phosphates

In both the CHO and the NIH-3T3 cells a high level of constitutive phosphoserine containing proteins did not permit an unequivocal detection of changes induced by the tested cyclic phosphates. However, as seen in Fig. 7, a marked augmentation in threonine phosphorylation induced by the six membered ring cyclic phosphates could be clearly detected in three specific proteins (18 kD, 35 kD, 38 kD). As shown, at 25°C, the bands at molecular weight of about 35 kD and about 38 kD reached a maximum phosphorylation at around 7 mins. which was slowly diminished while the band at about 18 kD displayed a much sharper phosphorylation-dephosphorylation profile. The presence of 5 mM phosphothreonine in the antibody binding assay reduced the intensity of the 35 KD band to the control level.

Example 22 Stability of the cyclic glycerophosphates

In principle, cyclic phosphates can be hydrolyzed to the respective linear forms (e.g. α-GP) either spontaneously or through putative phosphodiesterases. These possibilities were tested with the 4 cyclic phosphates (1,2 cGP, 1,3 cGP, 1,2 cPP and 1,3 cPPP (see Appendix A) in either aqueous solution or in cell lysates of CHO or NIH-3T3 cells (see Materials and Methods). 10 mg/ml cyclic phosphate in 1 ml of either PBS or PBS mixed with cell lysate (0.1 mg/ml protein) and incubated at 37° for up to 24 hours. Samples were tested at different times by thin layer chromatography on Silica gel 60 with n-propanol, concentrated ammonia, H2O (6:3:1 v/v/v). The R_f values for all 4 cyclic phosphates was in the range of 0.45-0.55 while α-GP and β-GP had R_f=0.14-0.16. This distinct difference allowed the qualitative detection of

hydrolysis of the cyclic phosphate. No hydrolysis of the tested cyclic phosphates either in PBS or by the cell lysate was detected. Furthermore, aqueous solutions of all the 6 cyclic phosphates were found to be stable for at least several days at room temperature and for months below 0°C.

Example 23 Inhibition of phosphatase and phosphodiesterase activity of cell lysates by cyclic phosphates

Inhibition of phosphatase activity of cell lysate by cyclic phosphates was assayed in 1.0 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 50 mM p-nitrophenyl phosphate, PNPP, as a substrate. Reaction was initiated by the addition of crude cell lysate (100 μg/ml final protein concentration) and terminated after 90 minutes at 37°C by an addition of 50 μl of 1M NaOH. The absorbance of the released p-nitrophenol was measured at 405 min. Assays were conducted under the same conditions as above in the presence of 50 μM cyclic phosphate. The enzyme activity in the absence of inhibitor was taken as 100% activity.

Only 1,3 cGP(E) obtained from PLase C cleavage of PG (see Materials and Methods) displayed a limited inhibition of the lysate phosphatases.

Synthetic 1,3 cGP, unlike the enzymatic product, displayed only approximately 10% inhibition at 50 μM concentration. Similarly, 50 μM 1,3 cPP displayed 15-20% inhibitory capacity. All other cyclic phosphates, as well as αGP and βGP at a concentration of 50 μM induced less than 5% inhibition of the cell lysate phosphatase activity.

In a series of analogous experiments phosphodiesterase activity of the cell lysates in the presence and absence of the cyclic phosphates was tested. In these experiments bis p-nitrophenyl phosphodiester was used as a substrate. No significant effect of any of the cyclic phosphates on the cell lysate phosphodiesterase activity was observed (not shown).

Example 24 Uptake of ^{32}P labeled 1,3-cPP by CHO cells

Binding and incorporation assays were carried out with ^{32}P labeled 1,3-cPP (1,3-c$P^{32}P$), the only one of the cyclic phosphates which could be synthesized in a relatively high specific activity (see Materials and Methods). When contacted with CHO cells there was a rapid apparent binding of 1,3-c$P^{32}P$ followed by a continuous uptake which leveled off after approximately 2 hours of incubation. A typical profile of binding and incorporation of 1,3-c$P^{32}P$ is shown in Figure 8. In this experiment a triplicate of 1 ml samples each containing $2x10^6$ CHO cells and 10 μM 1,3-c$P^{32}P$ (approximate specific activity 10 μci per μmole) were incubated in the starvation medium for different times. After 2 washings with PBS the cells were disintegrated in 1 M NaOH and radioactivity was scored with a β counter. The insert presents the magnified uptake profile for short incubations (up to 2 minutes) where the intercept (0 time incubation) corresponded to approximately $3x10^6$ molecules per cell, while at equilibrium (above 2 hours incubation) incorporation corresponded to approximately $2x10^8$ molecules per cell. This profile remained essentially unaltered in competition experiments where 1,3-c$P^{32}P$ was mixed with increasing amounts of unlabeled 1,3 -cPP or 1,3-cGP, indicating that the apparent binding extrapolated for 0 time incubation and the following incorporation proceeded through non-specific binding.

Without being limited to theory this mode of uptake may be explained by a mechanism by which 1,3-cPP and probably the other cyclic phosphates as well, first incorporate rapidly and non specifically into the cell plasma membrane (probably the lipid layer), where it reaches a steady-state concentration after a few seconds. Through this compartment the cyclic phosphate partitions further with the cytosol to reach an equilibrium with the external medium after approximately 2 hours. This passive partitioning mechanism is further supported by the estimated concentration of the intracellular concentration of 1,3 c$P^{32}P$ at the equilibrium which is in the micromolar range, i.e. the range of the external 1,3 c$P^{32}P$ concentration. It is of interest to note that after 5 minutes of incubation,

when tyrosine phosphorylation is maximal (see for example Figure 3) the intracellular concentration of the cyclic phosphate is in the order of $10^7 - 10^8$ molecules per cell.

Example 25 Intracellular binding of cyclic phosphates by CHO cells

In an attempt to characterize the intracellular binding of the cyclic phosphates, perforated CHO cells ($2x10^6$ in 1 ml of 1:1 v/v ethanol in PBS) were incubated for 5 minutes at 4°C with 20 μM 1,3-cP^{32}P (see above) and increasing amounts of either αGP, unlabeled 1,3-cPP or 1,3-cGP. Radioactivity was scored after 2 washings with PBS at 4°C as above. As shown in Figure 9, competition with unlabeled 1,3-cPP is displayed indicating specific binding. Similarly, clear binding competition was also observed with 1,3-cGP while αGP was ineffective. In this set of experiments the number of displaceable (i.e. specifically bound) ligands was estimated to be in the range of $10^7 - 10^8$ per cell. Such a high number suggests low affinity ubiquitous targets, rather than specific receptors.

Example 26 Effect of cyclic phosphates on differentiation of human breast cancer cells

The effect of the cyclic phosphates on the differentiation of human breast cancer cells was determined by detecting cell marker characteristics connected with differentiation. Highly undifferentiated breast cancer cells are characterized by low level of progesterone and estrogen receptors. Patients with such tumor have a poor prognosis. Partially differentiated breast cancer cells contain a significantly higher level of these receptors. Two available cell lines (established by Prof. Y. Kedar, Tel Aviv University, Tel Aviv, Israel) are used, which are of low and high estrogen and progesterone receptors, respectively. Cells are incubated in tissue culture medium with or without 1-10 μM cyclic phosphate or αGP (as a control). Change in receptor level in these cells is monitored by

conventional Western Blot analysis using known anti estrogen and anti progesterone receptor monoclonal antibodies.

Example 27 Effect of cyclic phosphates on differentiation of human T-leukemia cell lines

High virulent human T-leukemia cell lines (e.g. Jurkat cells) are cultured with various cyclic phosphates as above. The effect of the cyclic phosphates in these cells is monitored by the emergence of differentiation markers on the cell surface (e.g. CD3). Their quantity is determined by conventional FACS analysis using fluorescent antibodies.

Example 28 Effect of 1,3,cPP in combination with insulin on phosphorylation of cells expressing the insulin receptor

Binding of insulin to its receptor on the cell surface results in rapid tyrosine phosphorylation on the 117 kD and 200 kD receptor proteins. This is the initial event in the overt functions of insulin (e.g. glucose uptake).

CHO-T cells expressing the insulin receptor (obtained from Prof. Y. Zick, The Weizmann Institute, Rehovot, Israel) were divided into four groups, each incubated for 20 mins. with one of the following:

Group 1: 1 nM insulin which results in maximal phosphorylation of the receptors.

Group 2: 0.1 nM insulin resulting in partial phosphorylation of the receptors, in situation analogous to refractory response such as occurs in IDDM Type 2.

Group 3: 0.1 nM insulin and 0.1 μM 1,3, cPP; and

Group 4: 0.1 μM 1,3, cPP only.

Tyrosine phosphorylation was determined using anti-phosphotyrosine antibody (as described above). Phosphorylation was determined in the two

protein bands of the insulin receptor having a molecular weight of 117 kD (Fig. 10A) and 200 kD (Fig. 10B).

As seen in Fig. 10, incubation of the insulin receptor expressing cells with a combination of insulin (0.1 nM) and 1,3 cPP almost doubled the phosphorylation obtained in the cells incubated with insulin only. Thus, the tested GC shows synergistic activity with insulin.

Example 29 Tyrosine phosphorylation of Erb-b2 receptor on CHO cells by cyclic glycerophosphate

CHO cells expressing the human growth factor receptor (HGF) Erb-b2 (prepared by Y. Yarden, Weizmann Institute, Rehovot, Israel) are divided into the following four groups:

Group 1: Cells incubated for about 20 mins. with HGF at a concentration resulting in maximal phosphorylation of the HGF receptor;

Group 2: Cells incubated with concentrations of HGF resulting in partial phosphorylation of the receptor;

Group 3: Cells incubated with a combination of HGF in the lower concentration and the tested cyclic glycerophosphate; and

Group 4: Cells incubated with the tested glycerophosphate alone.

The percent of tyrosine phosphorylation of Erb B2 is monitored using known antibodies as described above.

Example 30: Augmentation of estrogen receptor (ER) receptor expression in human ER^{mod} $T_{47}D$ cells

Tumor cells were incubated for 7 days in the absence or presence of 1 μM of 1,3 cGP. On each of the noted days, cells were harvested by trypsinization, washed and fixed by dryng in air. The preparations were then peroxidased and protein blocked. Following this, the cells were labeled with first antibody (mouse-anti-human ER) for 30 mins. at room temperature (RT). After

washing, cells were incubated with biotinylated second antibody for 10 min at RT. After washing, the cells were incubated with streptavidin conjugated to horse radish peroxidase and color reaction with the DAB reagent was performed. Background staining was done with hematoxylin (mayer) and the fixation process was completed with water, alcohol and finally xylene immersions. The slides were covered, sealed and examined by a pathologist in a blind fashion. Expression of the ER was scored on a scale of 0 to 4 pulses, where 4 pulses is the highest score.

As seen in Fig. 11, following 7 days of incubation of the cells with 1,3, cGP, the level of expression of ER on the cells was significantly higher than the level of expression of ER in cells incubated in growth medium alone. Thus, incubation of these malignant cells with this 1,3 cGP results in their differentiation.

Example 31: Inhibition of proliferation of $T_{47}D$ human breast cancer cells by 1,3-cPP

In this experiment, $8x10^4$ $T_{47}D$ (clone 11) human breast cancer cells were plated in sets of 6 in 96 well microtiter plates and titrated concentrations (1-50 μM final concentrations) of 1,3-cPP salt in complete medium were added to the cultures at the beginning of the experiment only in a total volume of 200 μl per well. Plates were incubated at 37°C over the course of 5 days, where 1 plate was pulsed with ^{3}H-thymidine (Sigma) overnight each day and subsequently frozen. All the plates were harvested together (Packard Micromate 196 Harvester, Merriden, CT) and scored on a 96 well plate reader (Packard Matrix 96, Merriden, CT).

As seen in Fig. 12, incubation of the $T_{47}D$ cells with 1,3,cPP at a concentration of 50 μM resulted in significant inhibition of their proliferation as compared with the proliferation of the cells which were grown in growth medium

only. The effect was observed after 1, 2 and 3 days of incubation (P values were 0.0370, 0.0192, and 0.0238 on days 1-3 respectively).

Example 32: Induction of differentiation of K562 leukemia cells by 1,3, cPP

K562 leukemia cells are able to differentiate to the erythroid lineage. Such differentiation is characterized by the cells' ability to synthesize hemoglobin. Sodium butyrate is a known differentiation agent of such leukemia cells.

In this example, the ability of 1,3 cPP, α-GP and sodium butyrate alone and in combination to cause differentiation of K562 cells was evaluated using the known benzidine assay.

K562 cells were incubated for a period of 4 days with the following:

(a) growth medium (control);

(b) 10 μM of 1,3 cPP;

(c) 10 μM of α-GP;

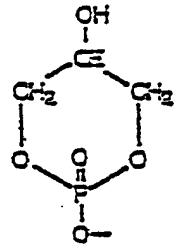
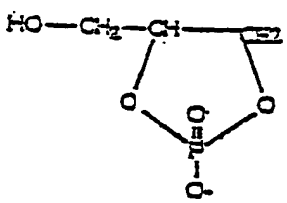
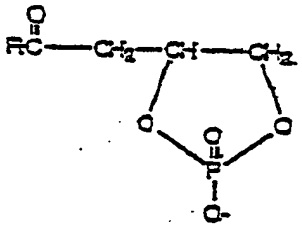
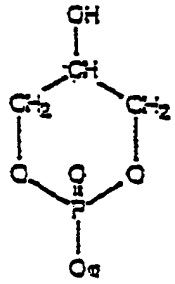
(d) 1.5 mM of sodium butyrate;

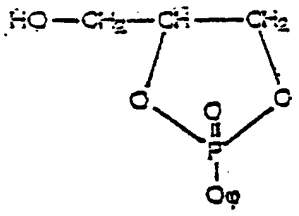
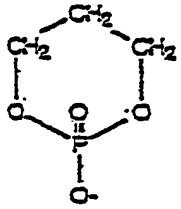
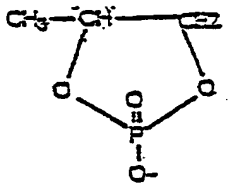
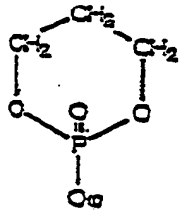
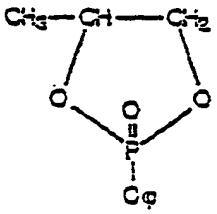
(e) a combination of sodium butyrate and 1,3 cPP; and

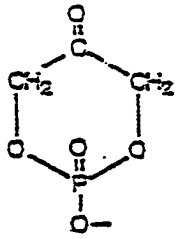
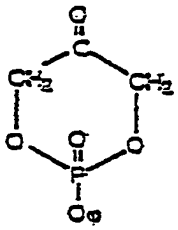
(f) sodium butyrate and α-GP.

As seen in Fig. 13, following a 4 day period of treatment, the synthesis of hemoglobin by the K562 cells which were incubated with a combination of sodium butyrate and 1,3 cPP was 3-fold greater than the level of hemoglobin synthesized by cells incubated with the differentiation inducing agent sodium butyrate alone. Thus, 1,3 cPP in combination with sodium butyrate caused significant differentiation of K562 leukemia cells to the erythroid lineage.

Appendix A

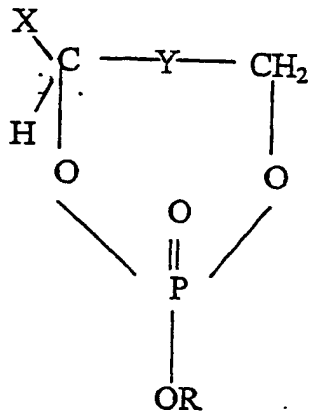
	Formula	Abbreviation
I		1,3 cGP
II		1,2 cGP
III		cyclic lysophosphatidic acid, c-lysoPA
IV		P-1,3 cGP

	Formula	Abbreviation
V		P-1,2 cGP
VI		1,3 cPP
VII		1,2 cPP
VIII		P-1,3 cPP
IX		P-1,2,cPP

	Formula	Abbreviation
X		cDHAP
XI		P-cDHAP

CLAIMS:

1. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general formula I:



wherein

Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl.

2. A pharmaceutical composition according to Claim 1, wherein said alkyl groups have 1-24 carbon atoms, said acyl groups are aliphatic saturated or unsaturated C_1 - C_{24} acyl groups and said aryl group is a carbocyclic aryl group optionally substituted by C_1 - C_4 alkyl, halogen and/or hydroxy.

3. A pharmaceutical composition according to Claim 2, wherein said acyl groups are derived from natural fatty acids.

4. A pharmaceutical composition according to Claim 3, wherein said acyl group is a saturated aliphatic acyl group selected from acetyl, butyryl, caproyl, octanoyl, decanoyl, lauroyl, myristyl, palmitoyl and stearoyl, or an unsaturated aliphatic acyl group selected from palmitoleyl, oleyl, linoleyl, and ricinoleyl.

5. A pharmaceutical composition according to any one of Claims 1-4, wherein said aryl group is phenyl.

6. A pharmaceutical composition according to Claim 1, comprising 1,2-cyclic propanediol phosphate.

7. A pharmaceutical composition according to Claim 1, comprising phenyl 1,2-cyclic propanediol phosphate.

8. A pharmaceutical composition according to Claim 1, comprising 1,2-cyclic glycerophosphate.

9. A pharmaceutical composition according to Claim 1, comprising phenyl 1,2-cyclic glycerophosphate.

10. A pharmaceutical composition according to Claim 1, comprising 3-acyl 1,2-cyclic glycerophosphate.

11. A pharmaceutical composition according to Claim 1, comprising cyclic oleyl lysophosphatidic acid.

12. A pharmaceutical composition according to Claim 1, comprising 1,3-cyclic propanediol phosphate.

13. A pharmaceutical composition according to Claim 1, comprising phenyl 1,3-cyclic propanediol phosphate.

14. A pharmaceutical composition according to Claim 1, comprising 1,3-cyclic glycerophosphate.

15. A pharmaceutical composition according to Claim,1 comprising phenyl 1,3-cyclic glycerophosphate.

16. A pharmaceutical composition according to Claim1, comprising cyclic dihydroxyacetone phosphate.

17. A pharmaceutical composition according to Claim1, comprising phenyl cyclic dihydroxyacetone phosphate.

18. A pharmaceutical composition for inducing phosphorylation in intracellular proteins of target cells comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of general Formula I of Claim 1.

19. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general Formula I of Claim 1 for promotion of cell differentiation in target cells.

20. A pharmaceutical composition according to Claim19, for the treatment of malignant diseases and disorders.

21. A pharmaceutical composition according to Claim20, wherein said malignant disorder is a blood malignancy.

22. A pharmaceutical composition according to Claim21, wherein said blood malignancy is leukemia.

23. A pharmaceutical composition according to Claim20, wherein said malignancy is breast cancer.

24. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general Formula1 of Claim 1, for induction of hormone-like signaling.

25. A pharmaceutical composition according to Claim24, wherein said hormone is insulin and the composition is for the treatment of non-insulin-dependent diabetes mellitus (non-IDDM type II diabetes).

26. A pharmaceutical composition according to Claim24, wherein said hormone is human growth hormone (HGH) for the treatment of disorders in which HGH is involved.

27. A pharmaceutical composition according to Claim24, wherein said hormone is epidermal growth factor (EGF) for the treatment of disorders involving EGF.

28. A compound of the general Formula I of Claim 1, with the exception of the following compounds:

i. compounds wherein Y is - $(CH_2)_m$ -, m is 0, X is CH_3, -CH_2OH or CH_2Oacyl wherein acyl is a saturated carboxylic acyl with more than 12 carbon atoms, and R is H or a cation;

ii. compounds wherein Y is - $(CH_2)_m$ -, m is 1, X is H and R is H , a cation or phenyl; and

iii. compounds wherein Y is – CH(OH) - , X is H and R is H, a cation or phenyl.

29. A compound according to Claim 28, selected from the group consisting of:

i. phenyl 1,2 cyclic glycerophosphate;

ii. phenyl 1,2 cyclic propanediol phosphate;

iii. cyclic dihydroxyacetone phosphate;

iv. phenyl cyclic dihydroxyacetone phosphate; and

v. cyclic oleyl lysophosphatidic acid.

30. A method for treatment of disorders and diseases which can be treated by phosphorylation of intracellular proteins comprising administering to the individual in need a therapeutically effective amount of a compound of general Formula I of Claim 1.

31. A method for the treatment of malignant diseases comprising administering to an individual in need a therapeutically effective amount of the compound of Formula I of Claim 1.

32. A method according to Claim 31, wherein said malignant disease or disorder is blood malignancy.

33. A method according to Claim 32, wherein said blood malignancy is leukemia.

34. A method according to Claim 31, wherein said malignant disease is breast cancer.

35. A method for the treatment of diseases involving hormone-like signaling comprising administering to an individual in need a therapeutically effective amount of the compound of Formula I of Claim 1.

36. A method according to Claim 35, wherein said hormone is insulin and the disease treated is non-IDDM type II diabetes.

37. A method according to Claim 35, wherein said hormone is human growth hormone (HGH) and the diseases treated are disorders in which HGH is involved.

38. A method according to Claim 35, wherein said hormone is epidermal growth factor (EGF) and the diseases treated are disorders involving EGF.

39. A method for detecting abnormal conditions of a tested cell comprising:

i. contacting the cells with cyclic glycerophosphates or their analogs (herein CGs) of formula I in Claim 1;

ii. detecting the level of phosphorylation in intracellular proteins of the tested cells; and

iii. comparing said level of phosphorylation to the level of phosphorylation in intracellular proteins of normal cells following contact with said CGs, a level of phosphorylation differing from that detected in the normal cells indicating a high probability of abnormality in the tested cells.

40. Use of a compound of the general Formula I as defined in Claim 1, for preparation of a medicament for the treatment of disorders and diseases that can be treated by phosphorylation of intracellular proteins.

41. Use of a compound of the general Formula I as defined in Claim 1, for the preparation of a medicament for the treatment of malignant diseases and disorders.

42. Use of a compound of the general Formula I as defined in Claim 1, for the preparation of a medicament for the treatment of diseases or disorders involving hormone-like signaling.

43. Use of a compound according to Claim 28, in the preparation of a medicament.

44. Use of a compound according to Claim 29, in the preparation of a medicament.

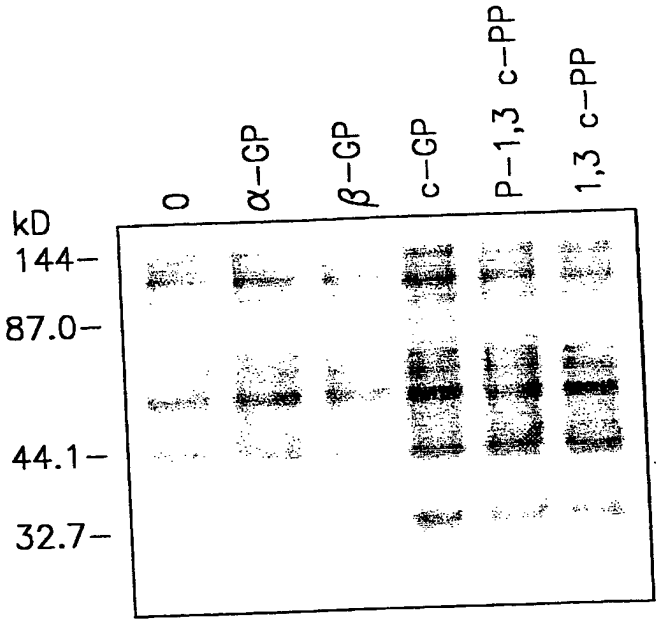

0
α-GP
β-GP
c-GP
P-1,3 c-PP
1,3 c-PP
kD
144-
87.0-
44.1-
32.7-

FIG.1

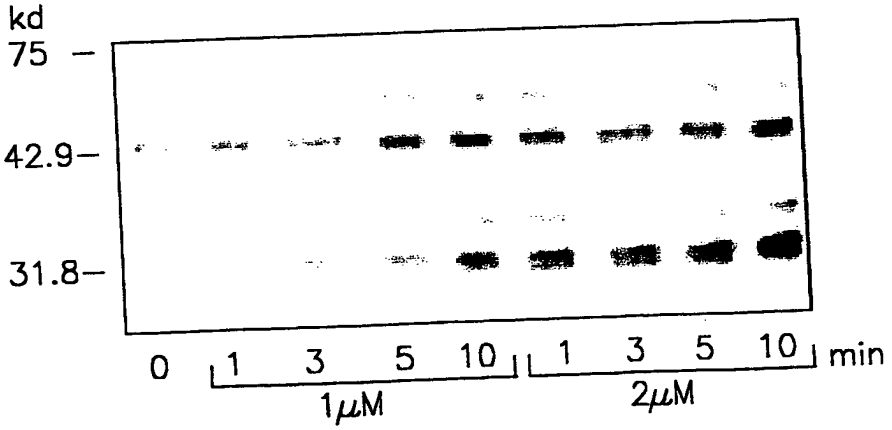

kd
75 -
42.9-
31.8-
0
1
3
5
10
1
3
5
10
min
1μM
2μM

FIG.2

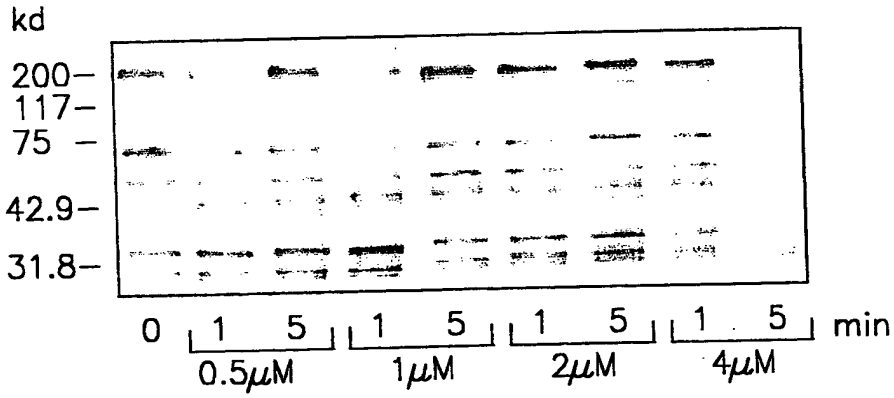

kd
200–
117–
75 –
42.9–
31.8–
0
1
5
1
5
1
5
1
5
min
0.5μM
1μM
2μM
4μM

FIG.3

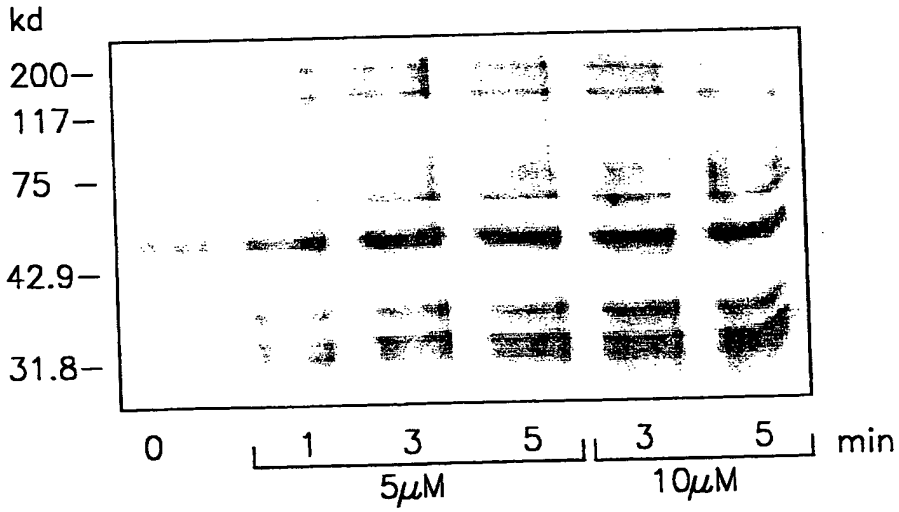

kd
200–
117–
75 –
42.9–
31.8–
0
1
3
5
3
5
min
5μM
10μM

FIG.4

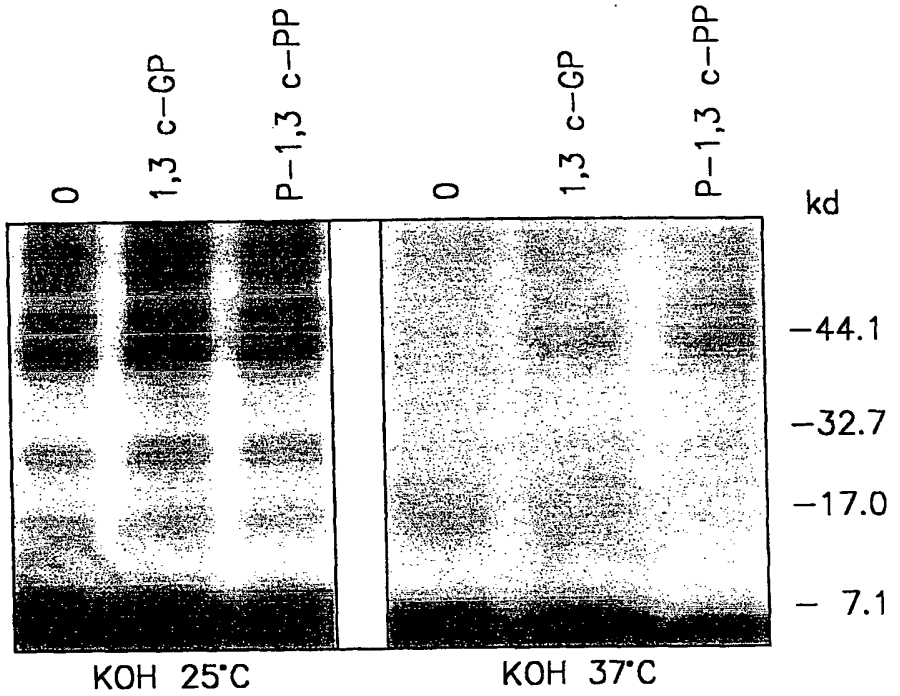

0
1,3 c-GP
P-1,3 c-PP
0
1,3 c-GP
P-1,3 c-PP
kd
-44.1
-32.7
-17.0
- 7.1
KOH 25°C
KOH 37°C

FIG.5

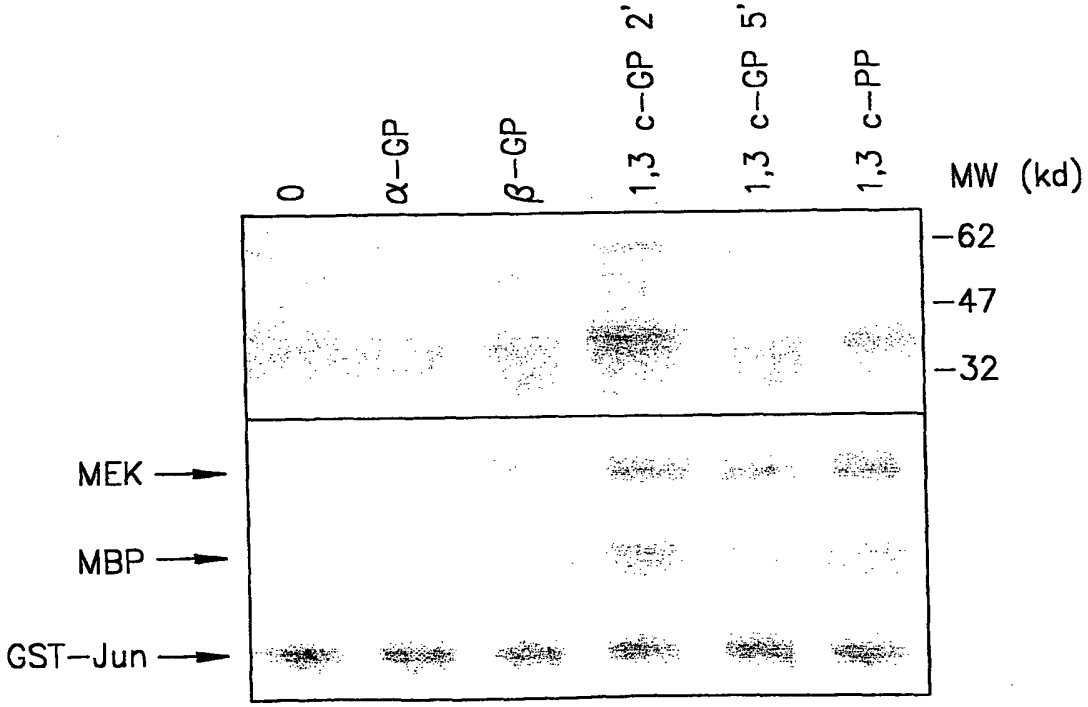

0
α-GP
β-GP
1,3 c-GP 2'
1,3 c-GP 5'
1,3 c-PP
MW (kd)
-62
-47
-32
MEK
MBP
GST-Jun

FIG.6

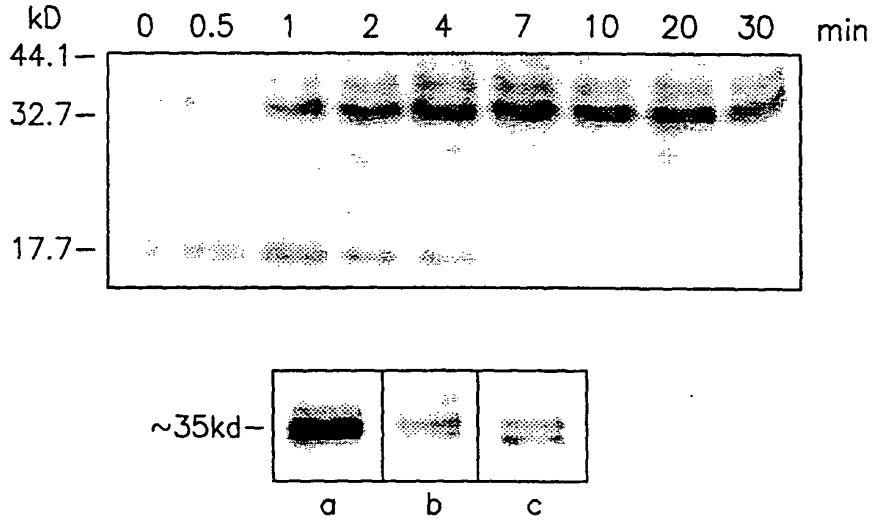

kD
0 0.5 1 2 4 7 10 20 30 min
44.1
32.7
17.7
~35kd
a b c

FIG.7

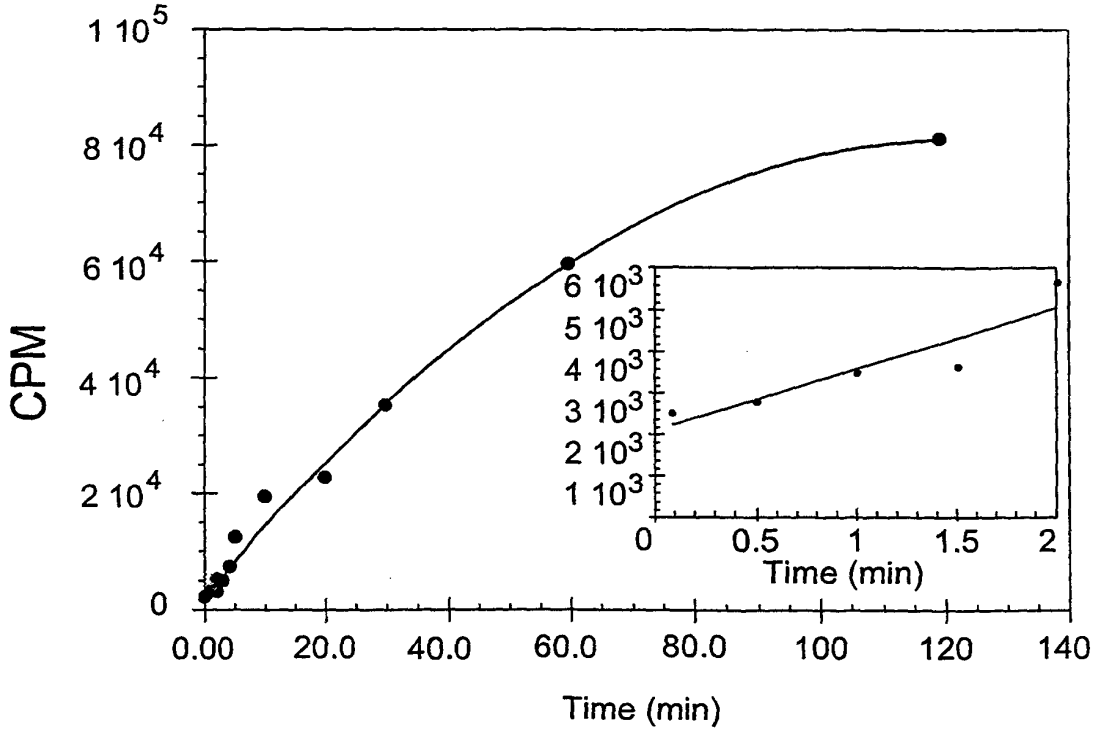

CPM
1 10^5
8 10^4
6 10^4
4 10^4
2 10^4
0
0.00 20.0 40.0 60.0 80.0 100 120 140
Time (min)
6 10^3
5 10^3
4 10^3
3 10^3
2 10^3
1 10^3
0 0.5 1 1.5 2
Time (min)

FIG. 8

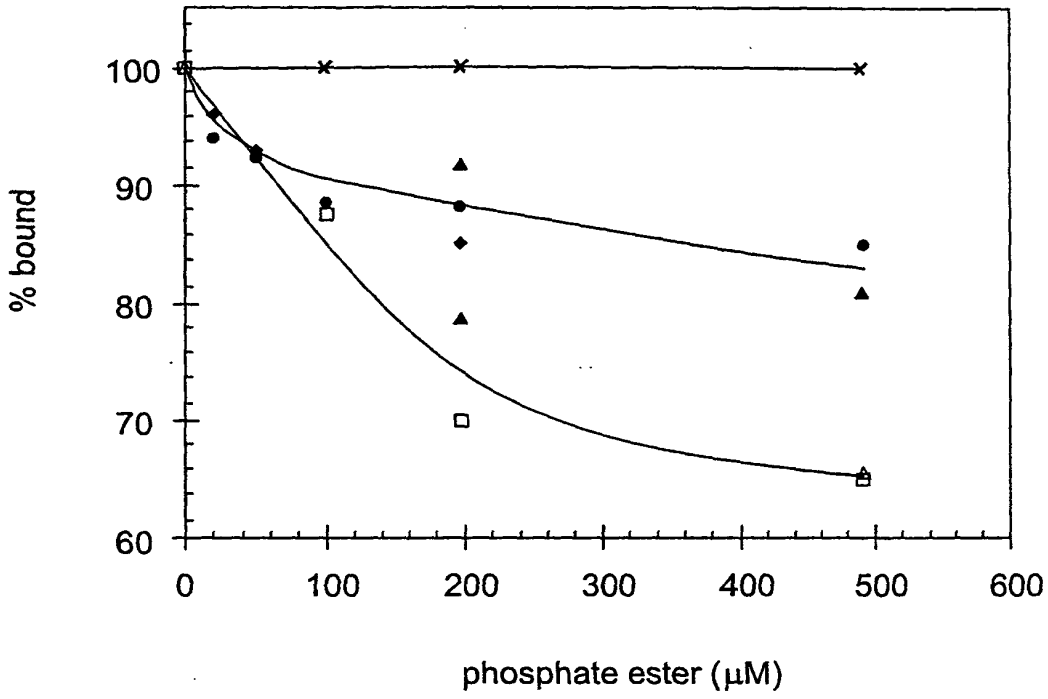

100
90
80
70
60
% bound
0
100
200
300
400
500
600
phosphate ester (μM)

FIG. 9

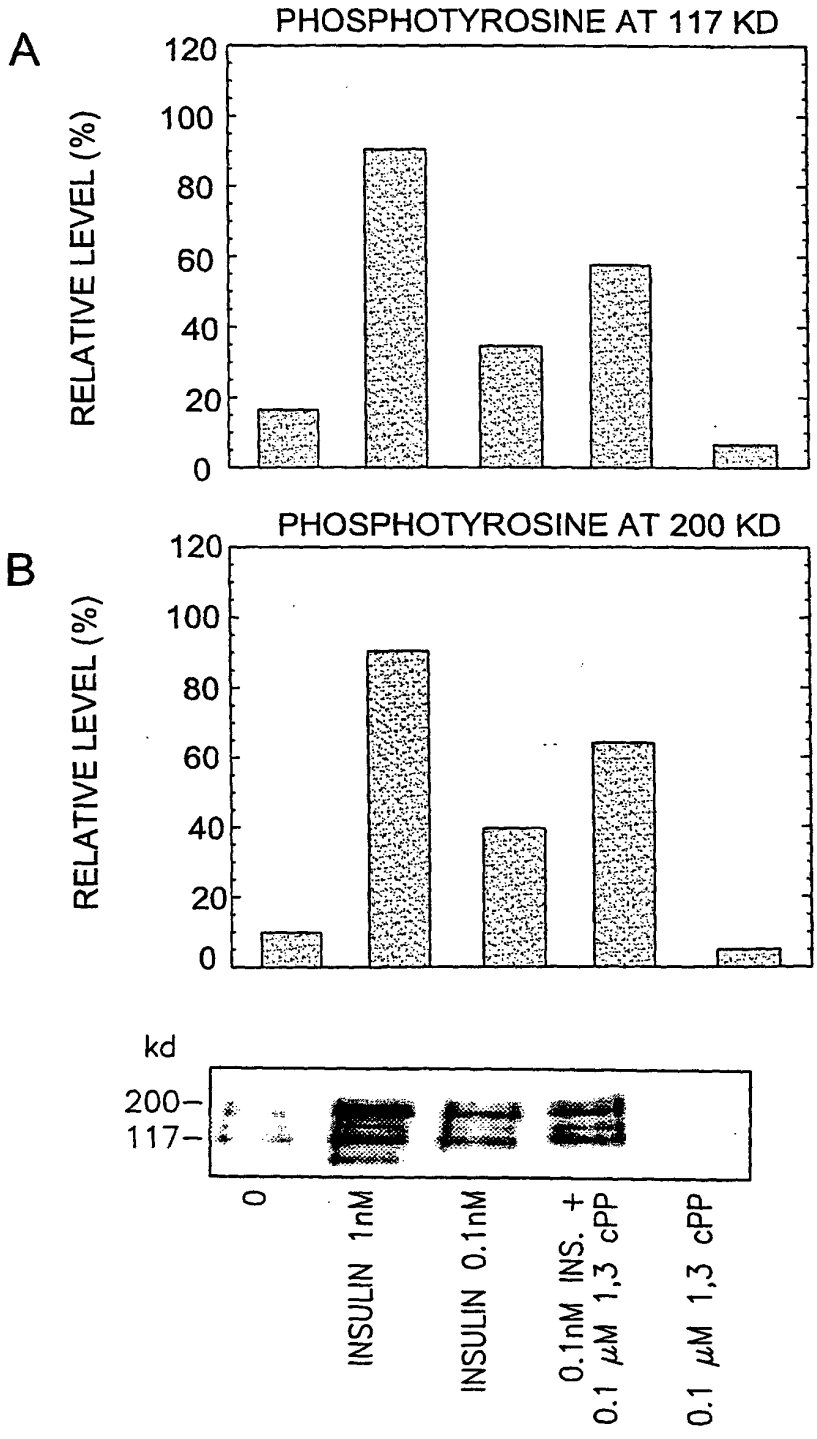

A
PHOSPHOTYROSINE AT 117 KD
RELATIVE LEVEL (%)
120
100
80
60
40
20
0
B
PHOSPHOTYROSINE AT 200 KD
RELATIVE LEVEL (%)
120
100
80
60
40
20
0
kd
200–
117–
0
INSULIN 1nM
INSULIN 0.1nM
0.1nM INS. + 0.1 μM 1,3 cPP
0.1 μM 1,3 cPP

FIG.10

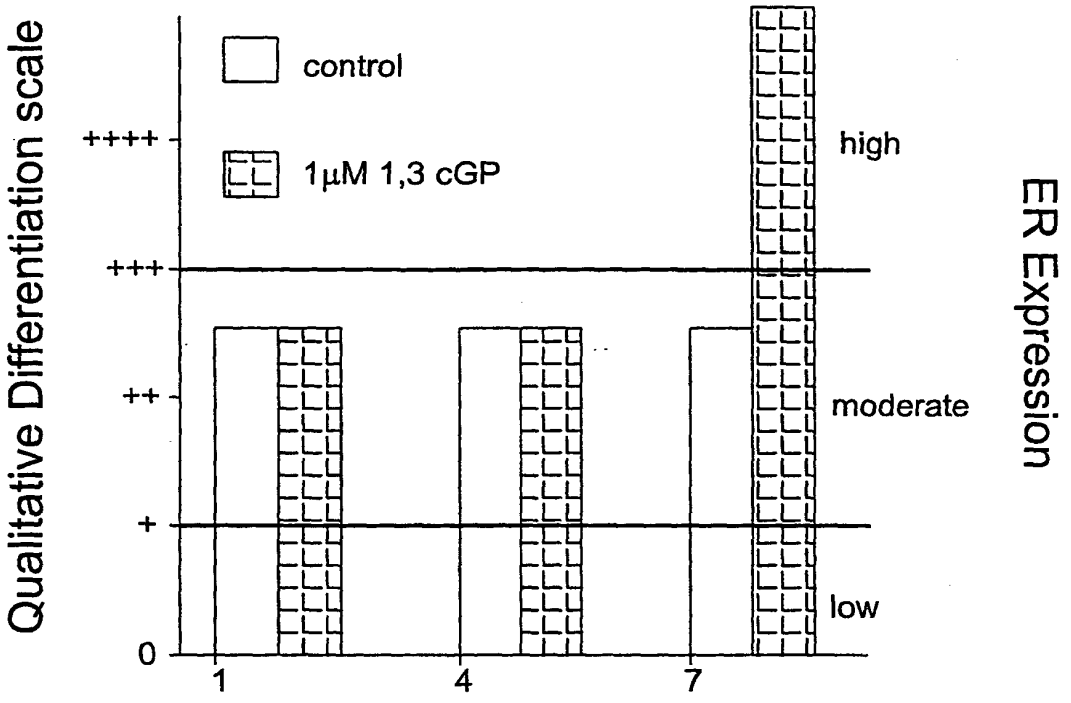

Qualitative Differentiation scale
control
1μM 1,3 cGP
++++
+++
++
+
0
1
4
7
high
moderate
low
ER Expression

FIG.11

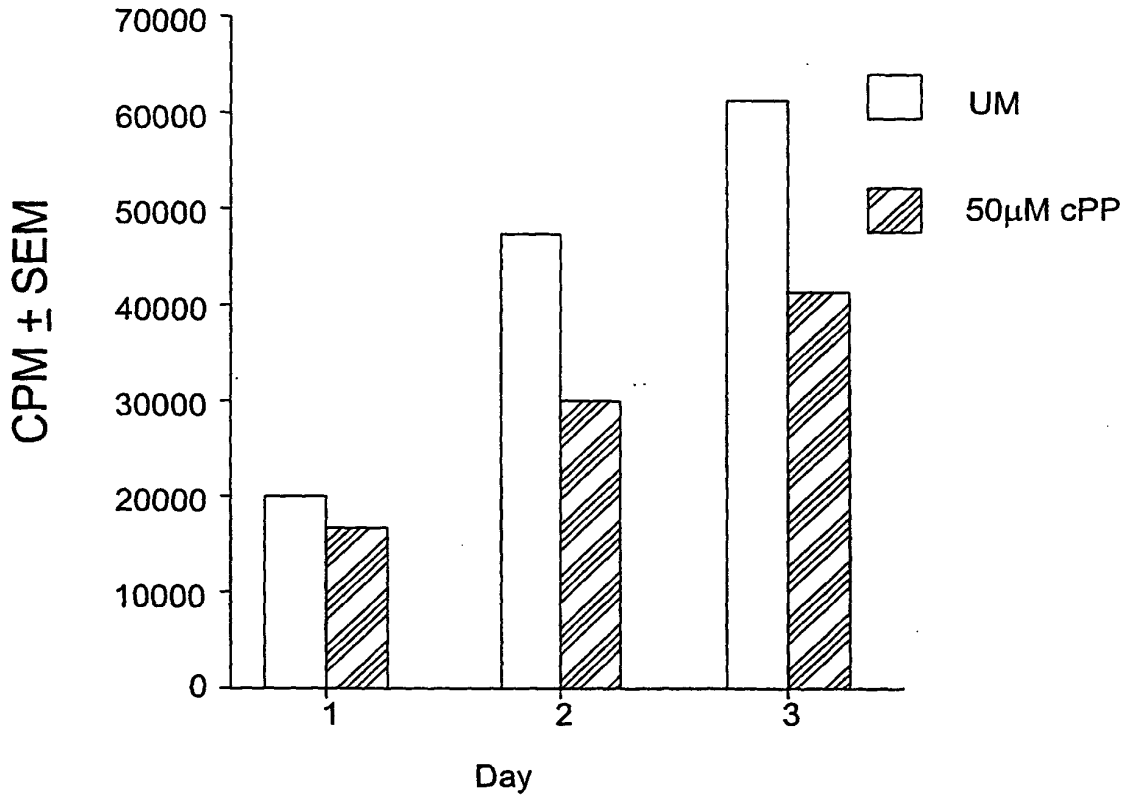

70000
60000
50000
40000
30000
20000
10000
0
CPM ± SEM
1
2
3
Day
UM
50μM cPP

FIG.12

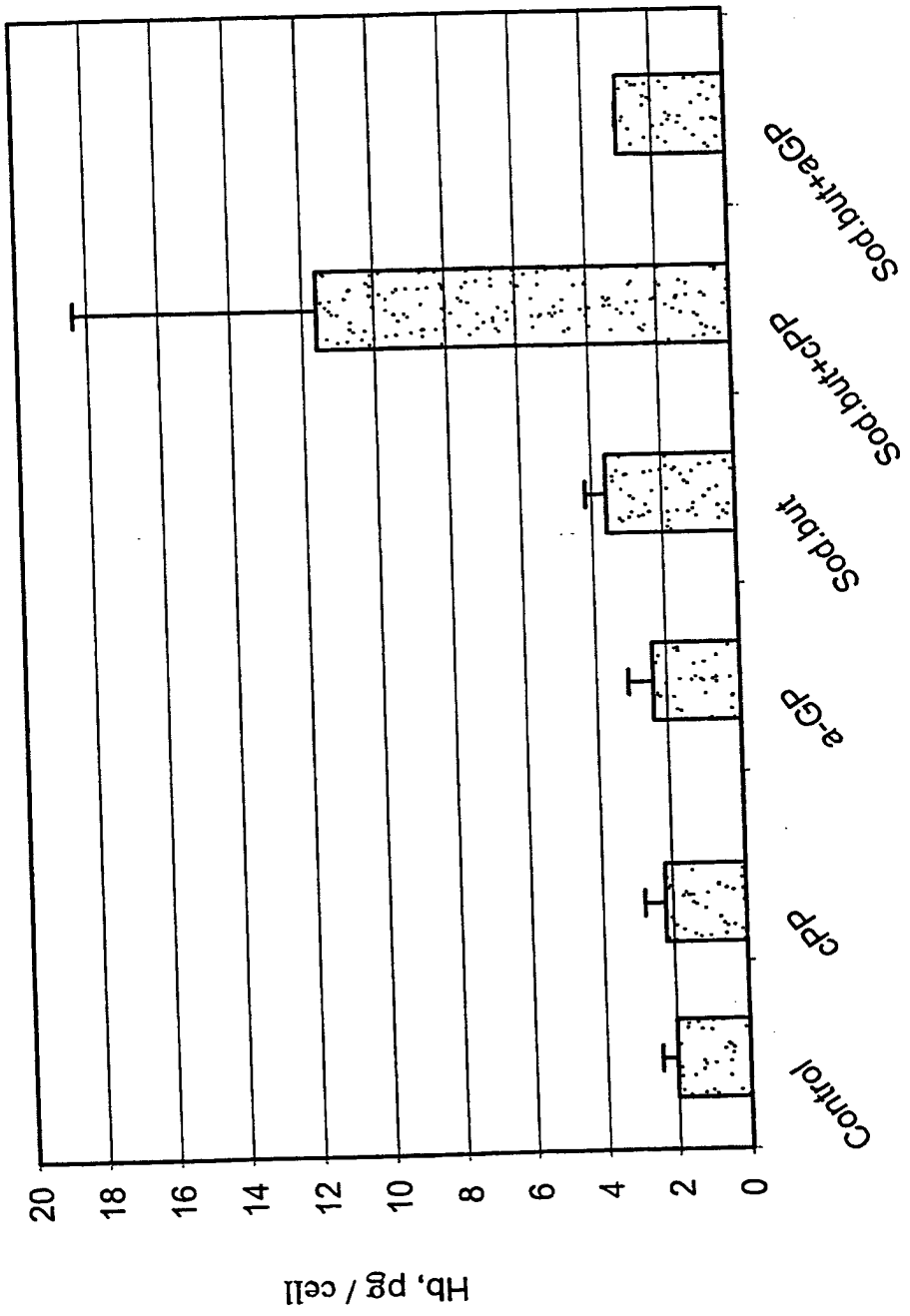

Hb, pg / cell
0
2
4
6
8
10
12
14
16
18
20
Control
CPP
a-GP
Sod.but
Sod.but+CPP
Sod.but+aGP

FIG.13

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IL 00/00184

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/665 A61P43/00 A61P35/00 A61P35/02 A61P3/10
C07F9/6574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 09139 A (ALLELIX BIOPHARMA ;BEGLEITER LEATH E (CA); WICKENS PHILIP L (CA);) 24 February 2000 (2000-02-24) abstract page 11, line 11 -page 12, line 10 page 13, line 26 -page 14, line 22; claims; example 1	1-4, 18-28, 30,35, 37,38, 40,42,43
P,X	MUKAI, MUTSUKO ET AL: "Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA)" INT. J. CANCER (1999), 81(6), 918-922 , XP000949280 the whole document	1-4, 18-28, 30-35, 37,38, 40-43

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 2000	16/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hoff, P

1

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Tumor metastasis inhibitors containing 1-O-acylglycerol-2,3-phosphates" retrieved from STN Database accession no. 126:220705 XP002148570 abstract & JP 09 025235 A (SAGAMI CHEM RES, JAPAN) 28 January 1997 (1997-01-28)	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506 XP002148571 abstract & JP 07 258278 A (SAGAMI CHEM RES, JAPAN) 9 October 1995 (1995-10-09)	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Method for preparation of 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:144502 XP002148572 abstract & JP 06 228169 A (SAGAMI CHEM RES, JAPAN) 16 August 1994 (1994-08-16)	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Promoters of protein phosphokinase C activation containing 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:350234 XP002148573 abstract & JP 07 149772 A (SAGAMI CHEM RES, JAPAN) 13 June 1995 (1995-06-13)	1-4, 18-28, 30, 35-40, 42,43

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 565 439 A (PIAZZA GARY A ET AL) 15 October 1996 (1996-10-15) abstract column 1, line 60 -column 2, line 39; claims; example II	1-4, 18-27, 30-35, 37,38, 40-42
X	D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300 see compounds (VI) and (VII) pages 1111 and 1114	1,5,7, 13,18, 19,24, 28,29
X	REVEL, MONIQUE ET AL: "Phosphorus heterocycles. XXVII. NMR study of 4-monosubstituted 1,3,2-dioxa- and -dithiaphospholane derivatives" ORG. MAGN. RESON. (1976), 8(8), 399-406 , XP000949315 page 399	1,5,7, 18,19, 24,28,29
X	SHINITZKY M ET AL: "Formation of 1,3-cyclic glycerophosphate by the action of phospholipase C on phosphatidylglycerol." JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 JUL 5) 268 (19) 14109-15. , XP000946147 figures 6,10	1,12,14, 16,18, 19,24, 28,29
X	T. UKITA ET AL.: "Organic Phosphates. I. Synthesis of 1,2-Diol Cyclic Phosphates." PHARM. BULL., vol. 5, 1957, pages 121-126, XP000949388 see compounds (I) and (V) pages 122-124	1,6,8, 18,19,24
X	SU, BANGYING ET AL: "Identification of a putative tumor marker in breast and colon cancer" CANCER RES. (1993), 53(8), 1751-4 , XP000946184 figure 4	1,8,18, 19,24
X	ABBOTT, STEVEN J. ET AL: "Chiral '160, 170, 180!phosphate monoesters. 1. Asymmetric synthesis and stereochemical analysis of '1(R)-160, 170, 180!phospho-(S)-propane-1,2-diol" J. AM. CHEM. SOC. (1978), 100(8), 2558-60 , XP000946182 see scheme II page 2558	1,6,18, 19,24

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 October 2000 (05.10.2000)

PCT

(10) International Publication Number
WO 00/57864 A3

(51) International Patent Classification[7]: A61K 31/665, A61P 43/00, 35/00, 35/02, 3/10, C07F 9/6574

(21) International Application Number: PCT/IL00/00184

(22) International Filing Date: 24 March 2000 (24.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
129179 25 March 1999 (25.03.1999) IL

(71) Applicant *(for all designated States except US)*: YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: SHINITZKY, Meir [IL/IL]; Derech Haganim 20, 46910 Kfar Shmaryahu (IL).

(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

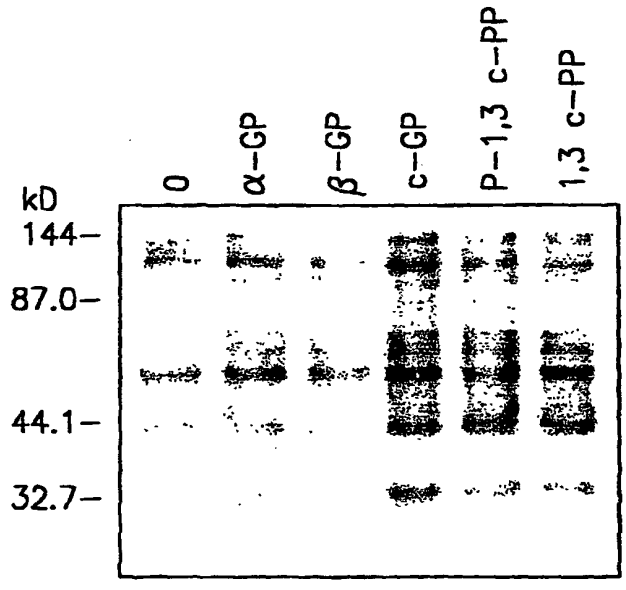
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*

(88) Date of publication of the international search report:
31 May 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CYCLIC GLYCEROPHOSPHATES AND ANALOGS THEREOF



(57) Abstract: Cyclic glycerophosphates as well as some analogs thereof (CGs) are shown to increase phosphorylation of intracellular proteins in various cells. Such activity is not found with linear α or β glycerophosphates. The phospharylating activity of the CGs render them useful in the prevention and treatment of various disorders and diseases such as, for example, different kinds of malignancies as well as disorders involving hormone and hormone-like signaling. The CGs are also useful for promotion of target cell differentiation and for detection of abnormal conditions in target cells.

WO 00/57864 A3

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/IL 00/00184

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/665 A61P43/00 A61P35/00 A61P35/02 A61P3/10
C07F9/6574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 09139 A (ALLELIX BIOPHARMA ;BEGLEITER LEATH E (CA); WICKENS PHILIP L (CA);) 24 February 2000 (2000-02-24) abstract page 11, line 11 -page 12, line 10 page 13, line 26 -page 14, line 22; claims; example 1	1-4, 18-28, 30,35, 37,38, 40,42,43
P,X	MUKAI, MUTSUKO ET AL: "Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA)" INT. J. CANCER (1999), 81(6), 918-922 , XP000949280 the whole document	1-4, 18-28, 30-35, 37,38, 40-43

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 2000	16/10/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Hoff, P

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Tumor metastasis inhibitors containing 1-O-acylglycerol-2,3-phosphates" retrieved from STN Database accession no. 126:220705 XP002148570 abstract & JP 09 025235 A (SAGAMI CHEM RES, JAPAN) 28 January 1997 (1997-01-28) ---	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506 XP002148571 abstract & JP 07 258278 A (SAGAMI CHEM RES, JAPAN) 9 October 1995 (1995-10-09) ---	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Method for preparation of 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:144502 XP002148572 abstract & JP 06 228169 A (SAGAMI CHEM RES, JAPAN) 16 August 1994 (1994-08-16) ---	1-4, 18-28, 30-35, 37,38, 40-43
X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Promoters of protein phosphokinase C activation containing 1-O-acylglycerol 2,3-cyclic phosphate" retrieved from STN Database accession no. 123:350234 XP002148573 abstract & JP 07 149772 A (SAGAMI CHEM RES, JAPAN) 13 June 1995 (1995-06-13) --- -/--	1-4, 18-28, 30, 35-40, 42,43

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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X	D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300 see compounds (VI) and (VII) pages 1111 and 1114	1,5,7, 13,18, 19,24, 28,29
X	REVEL, MONIQUE ET AL: "Phosphorus heterocycles. XXVII. NMR study of 4-monosubstituted 1,3,2-dioxa- and -dithiaphospholane derivatives" ORG. MAGN. RESON. (1976), 8(8), 399-406 , XP000949315 page 399	1,5,7, 18,19, 24,28,29
X	SHINITZKY M ET AL: "Formation of 1,3-cyclic glycerophosphate by the action of phospholipase C on phosphatidylglycerol." JOURNAL OF BIOLOGICAL CHEMISTRY, (1993 JUL 5) 268 (19) 14109-15. , XP000946147 figures 6,10	1,12,14, 16,18, 19,24, 28,29
X	T. UKITA ET AL.: "Organic Phosphates. I. Synthesis of 1,2-Diol Cyclic Phosphates." PHARM. BULL., vol. 5, 1957, pages 121-126, XP000949388 see compounds (I) and (V) pages 122-124	1,6,8, 18,19,24
X	SU, BANGYING ET AL: "Identification of a putative tumor marker in breast and colon cancer" CANCER RES. (1993), 53(8), 1751-4 , XP000946184 figure 4	1,8,18, 19,24
X	ABBOTT, STEVEN J. ET AL: "Chiral '160, 170, 180!phosphate monoesters. 1. Asymmetric synthesis and stereochemical analysis of '1(R)-160, 170, 180!phospho-(S)-propane-1,2-diol" J. AM. CHEM. SOC. (1978), 100(8), 2558-60 , XP000946182 see scheme II page 2558	1,6,18, 19,24

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte[illegible]nal Application No
PCT/IL 00/00184

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0009139 A	24-02-2000	AU 5473599 A	06-03-2000
JP 9025235 A	28-01-1997	NONE	
JP 7258278 A	09-10-1995	NONE	
JP 6228169 A	16-08-1994	NONE	
JP 7149772 A	13-06-1995	NONE	
US 5565439 A	15-10-1996	NONE	

09/937,386

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 October 2000 (05.10.2000)

PCT

(10) International Publication Number
WO 00/57865 A3

(51) International Patent Classification[7]: A61K 31/665, A61P 43/00, 35/00, 35/02, 3/10, C07F 9/6574

(21) International Application Number: PCT/IL00/00185

(22) International Filing Date: 24 March 2000 (24.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
129178 25 March 1999 (25.03.1999) IL

(71) Applicant *(for all designated States except US)*: YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; at the Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: SHINITZKY, Meir [IL/IL]; 20 Derech Haganim Street, 46910 Kfar Shmaryahu (IL).

(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
28 June 2001

(48) Date of publication of this corrected version:
15 November 2001

(15) Information about Correction:
see PCT Gazette No. 46/2001 of 15 November 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/57865 A3

(54) Title: PHARMACEUTICAL COMPOSITIONS COMPRISING CYCLIC GLYCEROPHOSPHATES AND ANALOGS THEREOF FOR PROMOTING NEURAL CELL DIFFERENTIATION

(57) Abstract: Cyclic glycerophosphates and analogs thereof (CGs) are shown to exert neural promoting activities in target cells. Such activities include promotion of neuronal outgrowth, promotion of nerve growth, provision of dopaminotrophic supporting envrionment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue. These activities of the CGs render them useful for treatment of various disorders including but not limited to mental disorders such as, for example, schizophrenia, dementia or disorders resulting in learning disablities. In addition, these CGs may be used for the treatment of neurodegenerative conditions such as Altzheimer's diesease, Parkinson's disease, conditions resulting from exposure to harmful environmental factors or resulting from a mechanical injury. The CGs may also be used to treat an individual suffering from a primary neurodengenerative condition in order to prevent or reduce the appearance of secondary degeneration in additional nerves ("*nerve rescue*").

PHARMACEUTICAL COMPOSITIONS COMPRISING CYCLIC GLYCEROPHOSPHATES AND ANALOGS THEREOF FOR PROMOTING NEURAL CELL DIFFERENTIATION

FIELD OF THE INVENTION

The present invention concerns pharmaceutical compositions comprising cyclic glycerophosphates and analogs thereof and treatment of neural-associated conditions and disorders.

PRIOR ART

The following is a list of references which is intended for a better understanding of the background of the present invention.

Boyd, R.K., De Freitas, A.S.W., Hoyle, J., McCulloch, A.W., McInnes, A.G., Rogerson, A. and Walter, J.A., *J. Biol. Chem.*, **262**:12406-12408 (1987).

Clarke, N. and Dawson, R.M.C., *Biochem. J.*, **216**:867-874 (1976).

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Knusel, *et al., Proc. Natl., Acad. Sci.* USA, **88**:961-965 (1991).

Leloir, L.F., *Biochem. Biophys., J.*, **33**:186 (1951).

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Markham, R. and Smith, J.D., *Biochem. J.*, **52**:552- (1952).

Shinitzky, M., Friedman, P. and Haimovitz, R., Formation of 1,3-cyclic glycerophosphate by the action of phospholipase C on phosphatidylglycerol, *J. Biol. Chem.*, **268**:14109-14115 (1993).

Su, B., Kappler, F., Szwergold, B.S. and Brown, T.R., *Cancer Res.*, **53**:1751-1754, (1993).

Tomac, A., *et al., Nature*, **373**:335-339 (1995).

Ukita, T., Bates, N.A. and Carter, H.E., *J. Biol. Chem.*, **216**:867-874, (1955).

BACKGROUND OF THE INVENTION

L-α-glycerophosphate (αGP), a key constituent in phospholipid metabolism (Kennedy and Weiss, 1956), is abundant in most biological tissues (Dawson, 1958). β-Glycerophosphate (βGP) is a product of enzymatic (Ukita *et al.*, 1955) and alkaline (Clarke and Dawson, 1976) hydrolysis of phospholipids and is formed through the cyclic phosphodiester intermediate 1,2-cyclic glycerophosphate (1,2 cGP) (Ukita *et al.*, 1955; Clarke and Dawson, 1976). 1,2 cGP has been detected in algae species (Boyd *et al.*, 1987) as well as in human cancer tissues (Su et al., 1993). Similarly, αGP can in principle adopt the cyclic form 1,3-cyclic glycerophosphate (1,3 cGP). This compound has been shown to be formed as an intermediate in the phospholipase C hydrolysis of phosphatidyl glycerol (PG) (Shinitzky *et al.*, 1993) and upon further hydrolysis is converted to αGP.

A six-membered cyclic phosphate of foremost biological importance is cyclic AMP. The ring of cyclic AMP is actually a derivative of 1,3 cGP backbone. Other cyclic phosphates which were detected in biological systems

include glucose cyclic phosphodiester (Leloir, 1951), 2',3'-cyclic phosphodiester (Markham and Smith, 1952), riboflavin-4',5'-cyclic phosphodiester (Forrest and Todd, 1950), myoinositol-1,2-cyclic phosphodiester (Dawson *et al.*, 1971) and cyclic lysophosphatidic acid (Friedman *et al.*, 1996).

Except for cyclic AMP and cyclic GMP which have been extensively studied, no specific biological activities have been so far assigned to the other biological cyclic phosphates.

There are several kinds of disorders and diseases which result from deterioration of areas of the brain and loss of neurons. One example of such diseases are neurodegenerative diseases such as Parkinson's disease (PD). Such diseases often involve degeneration of dopamine-producing neurons. Current therapeutic methods are mostly aimed at continuous stimulation of dopamine receptors by drugs which, although initially providing symptomatic relief, gradually lose effectiveness. Furthermore, such drugs do not prevent the progressive degeneration of dopaminergic neurons characteristics of such diseases.

A large number of growth factors such as nerve growth factor (NGF), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), insulin-like growth factor, brain derived growth factor and glial derived neurotrophic factor (Knusel B., *et al.*, 1990; Knusel *et al.*, 1991; Linn *et al.*, 1993) stimulate dopaminergic neuron survival and differentiation *in vitro*. In animal models involving induction of Parkinson's disease, the induced animals show improved behavior and an increase in tyrosine hydroxylase (TH), the key enzyme in the dopamine production pathway immunoreactviity when treated with factors like GDNF (Tomac, A. *et al.* 1995) and ciliary neurotrophic factor (CNTF) (Hagg, T. and Varon 1993).

List of compounds and their abbreviations

The following compounds which formulas are presented in **Appendix A** just before the claims, will be represented herein in the specification by their abbreviations as follows:

1. 1,3 cyclic glycerophosphate - **1,3 cGP**
2. 1,2 cyclic glycerophosphate - **1,2 cGP**
3. 3-acyl 1,2 cyclic glycerophosphate (cyclic lysophosphatidic acid) - **c-lysoPA**
4. Phenyl 1,3 cGP – **P-1,3 cGP**
5. Phenyl 1,2 cGP – **P-1,2 cGP**
6. 1,3 cyclic propanediol phosphate – **1,3 cPP**
7. 1,2 cyclic propanediol phosphate – **1,2 cPP**
8. Phenyl 1,3 cPP – **P-1,3 cPP**
9. Phenyl 1,2, cyclic propanediol phosphate – **P-1,2, cPP**
10. Cyclic dihydroxyacetone phosphate – **cDHAP**
11. Phenyl cyclic dihydroxyacetone phosphate - **P-cDHAP**

GLOSSARY

The following is an explanation of some terms used above and in the following description and claims:

CG – the cyclic glycerophosphates and analogs thereof used in the present invention.

Promoting neural cell differentiation – this term relates to the capability of the CGs used in the invention to cause cells to mature into neural cells after contact therewith. Such activity may be assessed by one of many *in vitro* and *in vivo* assays such as those described in the examples below. An example for an *in vitro* assay would be to grow cells capable of differentiating into nerve cells (e.g. PC12 cells) in the presence of a tested compound and to determine nerve outgrowth in

the cells by microscopic evaluation. *In vivo* assays may, for example, involve treatment of animals with injured dopaminergic neurons with the tested compounds and testing of motional and limb tremor parameters as well as *in situ* determination of molecules associated with dopaminergic transmission in the treated animals.

Target cells – any cells which have the potential to mature into neural cells. Non-limiting examples of such cells are PC12 and primary brain cells.

Analog - relates to any compound which is derived from one of the cyclic glycerophosphates of the invention and which substantially maintains the activity of the cyclic phosphate from which it was derived, including, for example, deoxy analogs and phenyl esters of the cyclic glycerophosphates, preferably, deoxy analogs.

Substantially maintaining - this term relates to the capability of analogs to promote the activity carried out by the cyclic glycerophosphate from which they were derived to a certain extent. The analog's activity will be considered to be substantially maintained wherein the activity is 30% or above, preferably 50% or above, more preferably 70% or above, and most preferably 90% or above the level of the activity of the cyclic glycerophosphate.

Effective amount – wherein the method of the invention is intended for prevention of a non-desired condition, the term *"effective amount"* should then be understood as meaning an amount of the active compound which, when administered, results in the prevention of the appearance of the said condition. Prevention of such a condition, e.g. a neurodegenerative condition, may be required prior to the appearance of any symptoms of a disease, e.g. in individuals having a high disposition of developing the disease, or when the compositions are used for the treatment of nerve rescue which is expected after nerve injury. Wherein the

compositions or methods are intended for treatment of an ongoing non-desired condition, the term *"effective amount"* should then be understood as meaning an amount of the active compound which is effective in ameliorating or preventing the enhancement of the treated condition and related symptoms.

Neural promoting activity – this term encompasses a variety of neural related activities which may be promoted in target cells upon their contact with the CGs used in the invention. Such activities include but are not limited to promotion of nerve growth, provision of dopaminotrophic supporting environment in a diseased brain, prevention of nerve degeneration, and nerve rescue.

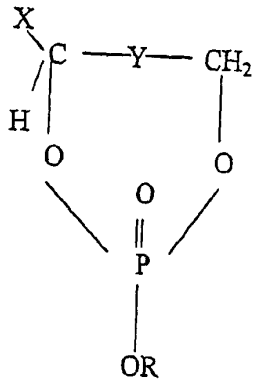
Prevention or treatment – the term prevention of disorders and diseases is to be understood in accordance with the invention as a reduction in the probability of the appearance of such disorders and diseases in an individual having a high predisposition of developing such disorders and diseases, reducing the extent of the symptoms associated with such disorders and diseases when they occur or completely preventing their appearance.

Treatment of such disorders or diseases in accordance with the invention means ameliorating the symptoms associated with the disorders or diseases, reducing the extent of such symptoms or completely eliminating them.

SUMMARY OF THE INVENTION

In accordance with the invention it has surprisingly been found that 1,2 cGP, 1,3 cGP and some of their analogs are capable of promoting neuronal outgrowth of PC12 adrenal tumorigenic cells in culture after a short incubation period.

The present invention thus provides, by a first of its aspects, a pharmaceutical composition for promoting neural cell differentiation in target cells comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general formula I:



(I)

wherein

Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl.

As used herein the term *"alkyl"* refers to an alkyl group having from about 1 to about 24 carbon atoms, e.g. preferably from about 3 carbon atoms to about 20 carbon atoms, most preferably from about 5 carbon atoms to about 15 carbon atoms; the term *"acyl"* refers to an aliphatic saturated or unsaturated C_1 - C_{24} acyl group, preferably an acyl group having an even number of carbon atoms, most preferably an acyl group derived from a natural fatty acid such as a saturated aliphatic acyl group selected from acetyl, butyryl, caproyl, octanoyl, decanoyl, lauroyl, myristyl, palmitoyl and stearoyl, or an unsaturated aliphatic acyl group selected from palmitoleyl, oleyl, linoleyl, and ricinoleyl; and the term *"aryl"* refers to a mono- or poly-carbocyclic aryl group, most preferably phenyl, optionally substituted by C_1 - C_4 alkyl, halogen and/or hydroxy. R may be any physiologically suitable cation and is preferably Na^+.

In one embodiment, Y is –CH(OH)-, X is H and R is H or phenyl. According to this embodiment, the composition comprises 1,3 cyclic glycerophosphate (1,3 cGP) or phenyl 1,3 cyclic glycerophosphate (P-1,3 cGP).

In another embodiment, Y is –C(=O)-, X is H and R is H or phenyl. According to this embodiment, the composition comprises cyclic

dihydroxyacetone phosphate (cDHAP) or phenyl cyclic dihydroxyacetone phosphate (P-cDHAP).

In a further embodiment, Y is $-(CH_2)_m-$, m is 0, X is $-CH_2OH$ and R is H or phenyl. According to this embodiment, the composition comprises 1,2 cyclic glycerophosphate (1,2 cGP) or phenyl 1,2 cyclic glycerophosphate (P-1,2 cGP).

In still a further embodiment, Y is $-(CH_2)_m-$, m is 0, X is a $C_1 - C_{24}$ alkyl, preferably $-CH_3$, and R is a cation or phenyl. According to this embodiment, the composition comprises 1,2 cyclic propanediol phosphate (1,2 cPP) or phenyl 1,2 cyclic propanediol phosphate (P-1,2 cPP).

In yet still a further embodiment, Y is $-(CH_2)_m-$, m is 1, X is a $C_1 - C_{24}$ alkyl, preferably $-CH_3$, and R is a cation or phenyl. According to this embodiment, the composition comprises 1,3 cyclic propanediol phosphate (1,3 cPP) or phenyl 1,3 cyclic propanediol phosphate (P-1,3 cPP).

In yet another embodiment, Y is $-(CH_2)_m-$, m is 0, X is $-CH_2$ (C_1-C_{24})acyl, preferably oleyl, and R is a cation. According to this embodiment, the composition comprises 3-acyl- 1,2 cyclic glycerophosphate (cyclic lisophosphatidic acid – c-lyso PA).

The CGs used in the invention may exert one of many neural promoting activities including but not limited to promotion of neuronal outgrowth, promotion of nerve growth, provision of dopaminotrophic supporting environment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue. All these activities fall within the scope of neural promoting activity.

Thus, the present invention also provides a pharmaceutical composition for promoting neural activity comprising a pharmaceutical acceptable carrier and, as an active ingredient, a compound of the general formula I above.

The ability of the pharmaceutical compositions of the invention to promote neural cell differentiation and neuronal activity in one or more of the above ways renders them extremely useful for treatment of various disorders. Thus, the invention also provides a pharmaceutical composition comprising a

pharmaceutical acceptable carrier and, as an active ingredient, a compound of the general Formula I above, for the prevention or treatment of disorders and diseases which can be prevented or treated by promoting neural cell differentiation and/or neural activity.

Such disorders may be mental disorders such as, for example, schizophrenia or dementia or disorders resulting in learning disabilities.

In addition, the pharmaceutical compositions of the invention may also be used for the treatment of neurodegenerative conditions involving damage to dopaminergic neural cells. Examples of such conditions are Alzheimer's disease (AD) or Parkinson's disease (PD).

Additional neurodegenerative conditions which are within the scope of the present invention are such which result from exposure of an individual to harmful environmental factors such as hazardous chemicals, neurodegenerative conditions resulting from a mechanical injury (e.g. injury of the optical nerve resulting from contact of the eye with an abusive external factor), etc.

Furthermore, it is known that, following primary degeneration of nerves, additional nerves present in the vicinity of the degenerated nerves undergo secondary degeneration. Treatment of an individual suffering from a primary neurodegenerative condition may prevent or reduce the appearance of secondary degeneration in additional nerves present in the vicinity of the degenerated nerves. Such treatment, termed *"nerve rescue"* is also within the scope of the present invention.

By yet another of its aspects, the present invention provides a method for inducing promotion of neural cell differentiation of target cells comprising contacting said target cells for a suitable period of time with an effective amount of a compound of the general formula I above.

A suitable period of time is such a period which enables the compositions of the invention to exert their activity. This period of time may easily be determined by a person skilled in the art for each kind of composition and target cells using any of the methods described herewith. Typically, and in contrast to

some known factors which affect neural cells such as NGF, the period of time required for the CGs used in the invention to be in contact with the target cells in order to exert their effect is very short (several minutes).

In accordance with an additional aspect of the invention, a method is provided for promoting neural activity in an individual comprising administering to the individual in need an effective amount of a compound of the general Formula I above.

A method for the prevention or treatment of disorders and diseases which can be prevented or treated by promoting neural cell differentiation and/or neural activity is also provided comprising administering to a person in need a therapeutically effective amount of a compound of Formula I above.

The method of the invention may be used for the treatment of a variety of disorders and diseases in which the abovementioned effects are beneficial, i.e., in which the effect of the CGs ameliorates or reduces the undesired symptoms of the treated condition or disease. These conditions and disorders may be for example, but not limited to, mental disorders such as schizophrenia or dementia, disorders leading to learning disabilities, neurodegenerative disorders such as Alzheimer or Parkinson disease and for prevention or treatment of nerve rescue following nerve injury.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1 shows photographs of PC12 cells following their incubation for 48 hours with growth medium containing linear α glycerophosphate as control (Fig. 1A), with nerve growth factor (NGF) at a concentration of 50 ng per/ml (Fig. 1B) and with 1,3 cyclic glycerophosphate (1,3 GP) at a concentration of 1 μM (Fig. 1C). Neuronal outgrowth is clearly seen in Figs. 1B and 1C.

Fig. 2 shows photographs of PC12 cells grown in culture medium (control) (Fig. 2A), pulsed for three hours with linear α and β glycerophosphates (Fig. 2B and 2C, respectively) with the cyclic glycerophosphates and analogs 1,3 cGP, phenyl-1,3 cGP, 1,2 cGP, 1,3 cPP, and phenyl-1,3 cPP (Fig. 2D – Fig. 2H

respectively) or with NGF (Fig. 2I). Following incubation, the cells were washed and grown in growth medium and the photographs show the cells on day 7 after treatment with the various factors. Neural outgrowth is seen only in PC12 cells treated with the above cyclic glycerophosphates and analogs (Figs. D-H).

Fig. 3 shows photographs showing PC12 cells treated for a period of 7 days control medium (Fig. 3A), αGP (Fig. 3B), βGP (Fig. 3C), the CGs: 1,3 cGP, phenyl-1,3 cGP, 1,2 cGP, 1,3 cPP, and phenyl-1,3 cPP (Fig. 2D – Fig. 2H respectively) at a concentration of 0.5 μM and with NGF (Fig. 3I). No neural outgrowth was observed after incubation with either α or β GP (Fig. 3B and C respectively) while neural outgrowth is observed to different extents in cells incubated with the various CGs (Fig. 3D-H). Under these conditions, extensive neural outgrowth is seen in cells grown with NGF (Fig. 3I).

Fig. 4 shows photographs showing PC12 cells treated for 14 days with control medium (4A), with 50 ng/ml NGF (4B), treated for 7 days with 50 ng/ml NGF and then washed and treated for another 7 days either with a growth medium without NGF (4C), or with 2 μM, 4 μM or 6 μM 1,3 cPP (4D, 4E, and 4F, respectively).

DETAILED DESCRIPTION OF THE INVENTION

Cyclic glycerophosphates can be formed by enzymatic degradation of phospholipids which in most cases yields five or six membered ring cyclic glycerophosphates. The present invention encompasses within its scope compositions comprising both such cyclic glycerophosphates formed by enzymatic degradation of phospholipids as well as synthetically formed ones. CGs having rings of less than five or more than six carbon atoms are also included within its scope.

The cyclic glycerophosphates and analogs thereof used in the invention may generally be synthesized using any one of the methods known in the art for synthesis of phosphate esters. Specific methods which may typically be used for

preparing the cyclic phosphates of the invention are described specifically below (see Examples).

Analogs of these cyclic glycerophosphates of the invention are also within the scope of the invention being typically deoxy analogs as well as phenyl esters of the 1,3 cyclic phosphates. These analogs may also be prepared by enzymatic methods or synthetically by any of the methods known in the art.

In addition to the active ingredient, the pharmaceutical compositions may also contain a carrier selected from any one of the carriers known in the art. The nature of the carrier will depend on the intended form of administration and indication for which the composition is used. The compositions may also comprise a number of additional ingredients such as diluents, lubricants, binders, preservatives, etc.

The compositions of the invention may be administered by any suitable way. A preferred mode of their administration is either i.v., topically or per os although at times it may be advantageous to use other administration modes as well.

Typically, the pharmaceutical compositions of the invention will comprise about 1 mg to about 10 mg of the active material per kg body weight of the treated individual.

While the compositions of the invention will typically contain a single CG, it is possible at times to include in the composition or to co-administer two or more CGs which may then act together in a synergistic or additive manner to prevent or treat the neurogenerative disorder.

The CGs used in the invention may be used in any of their isomer forms, (see for example, the four stereoisomers which constitute the synthetic 1,3 cGP depicted in Appendix A). For various purposes, one of the isomers may be preferred over the remaining ones.

According to the invention, the CGs may be administered either in a single dose or may be given repetitively over a period of time.

The compositions of the invention may also be administered to the treated individual in combination with an additional treatment, e.g. wherein the treated

condition is a neurodegenerative one, the compositions may be given together with one of the currently available drugs or therapies used for treatment of neurodenerative diseases such as dopamine receptor stimulants, L-dopa or together with a growth factor such as NGF. In such a combination treatment the CGs may be administered simultaneously with or at different times than the administration of the additional treatment so as to yield a maximum preventive or therapeutic effect.

EXAMPLES

The invention will now be illustrated by the following non-limiting examples with reference to the appended figures.

CHEMICAL SECTION

Synthesis of the cyclic phosphates

The cyclic phosphates of the invention are prepared by the reaction of a suitable dihydroxy compound wherein Y is - $(CH_2)_m$ - or - C(=O) - and X is H or alkyl with phosphorus oxycloride ($POCl_3$) when R is H or with aryl, e.g. phenyl, phosphorodichloridate (RO-P(=O)Cl_2) when R is aryl.

When there is one or more hydroxy groups in the starting compound, namely Y is - CH(OH) - and/or X is - CH_2OH -, these hydroxy groups have to be protected, e.g. by benzylation, and the benzyl group is then removed after cyclization by conventional catalytic hydrogenation in the presence of a suitable catalyst such as Pt or Pd.

The reaction is carried out in an anhydrous solvent, e.g. dioxane or methylene chloride, in the presence of equivalent amounts of a nucleophile such as pyridine or triethylamine. The end products, when R is not aryl, are usually obtained as salts.

The synthesis of a series of known and novel 5- and 6-membered ring cyclic phosphates is illustrated below.

Example 1: Synthesis of 1,3 cyclic glycerophosphate (1,3 cGP)

The procedure of Buchnea (Buchnea, 1973) was followed essentially as described. Briefly, 2-benzyloxy-1,3-propanediol (Aldrich) was reacted with an equimolar amount of phosphorus oxychloride (Aldrich) in methylene chloride. The resulting 2-benzyl-1,3 cGP was treated with hydrogen under the catalysis of Pd black in methanol to remove the benzyl residue. The 1,3 cGP, isolated as the Ba salt, was pure on paper chromatography (n-propanol: ammonia: water 6:3:1, R_f=0.52).

1,3 cGP was also produced by the cleavage of phosphatidyl glycerol (PG) with phospholipase C as described (Shinitzky et al., 1993). The product had a trace of approx. 10-20% α-GP as indicated by paper chromatography.

Example 2: Synthesis of 1,2 cyclic glycerophosphate (1,2 cGP)

This compound was prepared as described (Kugel, L. and Halmann, M., *J. Am. Chem. Soc.*, **89**:4125-4128 (1967). The disodium salt of β-glycerophosphate (Sigma) was first converted to the acid form and then cyclized with dicyclohexylcarbodiimide (Aldrich). The product, isolated as the Ba salt, was pure on paper chromatography.

Example 3: Synthesis of phenyl 1,3 cyclic glycerophosphate (P-1,3 cGP)

The method described in Example 1 for 1,3 cGP was followed by reacting 2-benzyloxy-1,3-propanediol with phenyl phosphorodichloridate (Aldrich). The intermediate benzylated product was pure on thin layer chromatography (ethyl acetate:hexane 3:2 R_f =0.58), with a melting point of 136°C. It was further hydrogenated as in Example 1 to remove selectively the benzyl residue. The obtained P-1,3 cGP, compound **III**, was pure on thin layer chromatography (as above) with R_f =0.15 and melting point of 116°C.

Example 4: Synthesis of 1,3 cyclic propanediol phosphate (1,3 cPP)

1,3 cPP was prepared by reacting 1,3-propanediol (Aldrich) with an equimolar amount of phosphorus oxychloride and then purified as described by

Buwalda et al., 1997. The product was isolated as the free acid (melting point: 99-100°C).

^{32}P labeled 1,3 cPP (1,3 $cP^{32}P$) was prepared with $^{32}POCl_3$. The latter was obtained by introducing a trace of $H_3{}^{32}PO_4$ (Amersham) into an excess of $POCl_3$ in the cold (Neuhaus and Korkes, 1958). The reaction was then proceeded on a microscale and 1,3 $cP^{32}P$ was isolated by co-crystallization with unlabelled 1,3 cPP.

Example 5: Synthesis of 1,2 cyclic propanediol phosphate (1,2 cPP)

1,2 cPP was prepared by the same procedure as in Example 4 but using 1,2- propanediol (Aldrich). The compound was isolated as the Ba salt and was pure on paper chromatography (n-propanol:ammonia:water 6:3:1, R_f=0.55).

Example 6: Synthesis of phenyl 1,3 cyclic propanediol phosphate (P-1,3 cPP)

P-1,3 cPP was prepared by a method analogous to the procedure of Example 4, by reaction of 1,3-propanediol with an equimolar amount of phenyl phosphorodichloridate in dry pyridine. The product was crystallized twice from ethyl acetate-hexane and had a melting point of 72°C.

Example 7: Synthesis of phenyl 1,2 cyclic glycerophosphate (P-1,2 cGP)

This novel compound was prepared as in Example 3 by reaction of 1-benzyloxy-2,3-propanediol with phenyl-PO_2Cl_2, followed by removal of the benzyl residue by selective hydrogenation.Crystallization was achieved from ethanol-acetone and the product had a melting point of 95°C.

Example 8: Synthesis of phenyl 1,2 cyclic propanediol phosphate (P-1,2 cPP)

This novel compound was prepared as in Example 6 by reaction of 1,2-propanediol with an equimolar amount of phenyl-PO_2Cl_2 in dry pyridine.

Crystallization was achieved from ethyll acetate-hexane and the product had a melting point of 69°C.

Example 9: Synthesis of cyclic dihydroxyacetone phosphate (cDHAP)

This novel compound was prepared by reaction of $POCl_3$ with dihydroxyacetone.

1.8 g (0.01M dimer or 0.02M monomer) Dihydroxyacetone dimer MW-180 dissolved in 20 ml fresh distilled methylene chloride.

3.07 g = 1.87 ml (0.02M) Phosphoryl chloride (MW-153.5, d-1.645) in 4 ml $MeCl_2$ was slowly added to the solution at RT. The solution was refluxed for 15 h (the solution was black). Methylene chloride was evaporated and 100 ml 90% acetone/water was added to the solution. The reaction mixture was refluxed for 18 h. The black solution was treated with active carbon at RT and filtered. From the resulting slightly yellow solution was evaporated acetone and water and the very nice crystalline residue was dissolved in 10 ml acetone. 0.01 M BaJ_2 in 80 ml acetone was added to the solution and nice crystals of cyclic-dihydroxyacetone-phosphate barium salt started to precipitate. The precipitate was washed 3 times with small quantities of acetone and dried. The product was cleaned by dissolving it in small amounts of water and precipitating with acetone. The resulting produce is white crystalline powder and shows in paper chromatography (solvents mixture: n-Propanol:NH_4H_2O 6:3:1) R_f – 0.50.

Example 10: Synthesis of phenyl cyclic dihydroxyacetone phosphate (P-cDHAP)

This novel compound was prepared by reaction of phenyl-PO_2Cl_2 with dihydroxyacetone in dry pyridine. Upon removal of the solvent by vacuum, the residue was extracted twice with ethyl acetate. After evaporation of the ethyl acetate, an oily residue was obtained..

Example 11: Synthesis of cyclic oleyl lysophosphatidic acids (c-lysoPA)

These novel compounds were prepared by reaction of oleyl lysophosphatidic acid (Avanti Polar Lipids) with excess dicyclohexylcarbodiimide (DCC) in dimethyl sulfoxide. The product appeared as a oil.

BIOLOGICAL SECTION

Materials and Methods

The immortal PC12 cell line is one of the most investigated systems in neuronal differentiation. In the presence of nerve growth factor these cells differentiate to neuronal cells. PC12 cells originated from rat pheochromocytoma were grown as monolayers in Eagle's medium (EM) supplemented with 10% fetal calf serum, 50 μg/ml gentamicin and 5 mM glutamine, in a humidified incubator buffered with 5% CO_2, at 37°C. The culture medium is changed every four days and the cells are passaged every eight days and performed confluent monolayers (1.5 x 10^6 in a 10 cm plate or 10^5 cells per well in 24 wells plate). PC12 cells are originally round cells which, following several days in the presence of nerve growth factor (NGF) process nerves. Upon withdrawal of the NGF, the nerves retract and a process of apoptosis is initiated in the cells.

Induction of PD in rats

Sprague-Dawley (SD) rats (weighing 230-250 g) are anesthetized with ketamine plus xylazine administered i.p. and their head secured in a stereotaxic frame. 6OH-DA (8 mg/4ml) is then injected into the median-forebrain-bundle to destroy the dopaminergic terminals unilaterally (Fitoussi, N., *et al. Neuroscience,* **85**(2):405-413) (1998)). Manifestations of the disease are evident within 2-3 weeks.

Dopaminergic ablation is assessed behaviorally using a rotometer test, which is based on upregulation of dopamine receptors on the lesioned side. Systemic administration of a DA agonist (apomorphine, 0.25 mg/kg s.c.) induces

rotation in rats with unilateral dopaminergic ablation, with rotation occurring in the direction contralateral to the side of the lesion.

Administration of cyclic glycerophosphates and analogs thereof into the brain

Cyclic phosphates are administered into the brain using ALZET osmotic pumps (ALZET Corporation, Palo Alto, CA). A canulla (30 gauge) is implanted 0.5 mm medial to the SN of rats, using a stereotaxic device after assessment of nigrostriatal lesions (rotation behavior). Cyclic phosphates are microperfused at a rate of 1 μl/h for 3 or 14 days.

Brain dissection and extraction

Rats are decapitated and their brains rapidly removed. The brains are then placed in a rat brain mold on ice and 0.5 mm serial sections are cut and placed on chilled microscope slides. Tissue punches are rapidly taken using a stainless steel cannula with an inner diameter of 1.1 mm, according to the following coordinates: A1.5-2.0 mm for the striatum; P5.5-5.0 mm for the SN, and include most of the desired regions. The tissue samples are immediately frozen in liquid nitrogen and stored at -70°C until extraction. Extraction is achieved by thawing the punches and subjecting them to probe sonication (80 watts for 5 sec. with a Sonifier B-12; Branson, Danbury, CN) in 0.5 ml of a perchlorate solution (0.1M) containing EDTA/ethanol (0.021%) on ice. A sample (100 μl) is removed for protein analysis and the remainder is centrifuged (2000 x g, 10 mins. 4°C). The resulting supernatants (the tissue extracts) are filtered (0.45 μm Acrodisk, Gelman; Ann. Arbor. MI) and stored at -70°C until subjected to ELISA analysis to determine ILS or GDNF or HPLC analysis to determine the 5-HT and 5-HIAA content.

Assessment of GDNF

The effect of cyclic glycerophosphates on the release and production of GDNF from the SVG-cells and brain tissue extracts is determined as follows. Cells are incubated for 12, 24 and 48 hours with or without cyclic phosphates. Supernatants are taken after centrifugation and analyzed for GDNF using an ELISA kit (ENDOGEN, MA, USA and PROMEGA, Madison, USA, respectively).

Isolation of RNA

Total RNA is isolated from cultured cells or tissue extracts, using Tri Reagent™ (Boehringer Mannheim, Germany). Cells are lysed in the reagent (10^6 cells/1 ml reagent). Frozen tissue punches are homogenized with the reagent (50 mg tissue/1 ml) using a glass Teflon rod. Chloroform is then added and the homogenates are separated into three phases by centrifugation. Care is taken when removing the aqueous phase so as not to disturb the interphase or the organic phase, In order to avoid genomic DNA contamination, RNA is precipitated from the aqeuous phase by addition of isopropanol, washed with ethanol and solubilized in DEPC treated water. RNA is estimated spectrophotometrically at 260 nm and 280 nm and stored at -80°C until use.

First strand cDNA synthesis is carried out in a reaction volume of 20 μl containing 3 μg of total RNA, 10 mM primer dT (Boehringer Mannheim, Germany) and 1 mM dNTP mix (Boehringer Mannheim, Germany). After heating for 2 min. at 65°C and cooling back to 4°C, the reaction is initiated by the addition of 50 units M-MuLV reverse transcriptase and 20 units RNAse inhibitor (Boehringer Mannheim, Germany). The mixture is then brought to 37°C for 60 mins. PCR on the cDNA was carried out in a reaction volume of 50 μl. First strand cDNA (2 μl) is added to the PCR mixture containing the following components: 0.2 mM dNTP mix (Boehringer Mannheim, Germany), 1 mM each oligonucleotide primer (primers were designed according to the published GDNF cDNA sequence. 5′-TCACCAGATAAACAAATGGC-3′{5′} and

5′-TACATCCACACCTTTTAGCG -3′{3′} corresponding to bases 81-101 and 460-480 respectively) (Biosource, CA, USA), and 2.5 U Taq DNA polymerase (Boehringer Mannheim). Reactions are overlaid with mineral oil, and initially denatured at 94°C for 2 min. PCR is performed using a MJ Research thermal cycler programmed for 40 cycles consisting of denaturation at 94°C for 1 min. followed by primer annealing at 55°C for 1 min. and primer extension at 72°C for 1 min. At the end of the 40 cycles, the reaction mixture is kept at 72°C for 10 min. The PCR product is electrophoretically analyzed on a 2% agarose gel containing ethidium bromide).

Immunohistochemical assessment of the cell survival in the brain

At the end of the experiment the animals are anesthetized with ketamine and xylazine i.p. and then perfused via cardiac puncture with PBS followed by 4% paraformaldehyde. The brains are then removed and post-fixed in 4% paraformaldehyde for 24 hrs and then transferred into 20% sucrose for 48 hours. Tissue sections of 35 μm are obtained using a cryostat and placed in 24 wells plate in PBS. The sections are incubated overnight in 4°C with a primary rabbit polyclonal antibody to Tyrosine hydroxylase (TH) (Chemicon, CA, USA) or a primary mouse monoclonal antibody to glial fibrillary acidic protein (GFAP) (Chemicon, CA, USA). The sections are then washed with PBS, incubated (1 hr) with a HRP conjugated secondary antibody (sheep anti-rabbit or anti-mouse) (Chemicon, CA, USA) and washed with PBS. Then, the sections are incubated with the chromagen diaminobenzidine (DAB), counter-stained with hematoxylin, and screened by light microscopy. Positive staining for TH indicates the amount of dopaminergic- cells in the striatum and substantia nigra, i.e. dopaminergic-cells survival. Positive staining for the GFAP in the injection tract indicates glioma processes.

Microdialysis

The microdialysis technique requires implantation of a small (500 μm diameter) probe into the brain of live rats. Implanation is performed on rats as follows. A rat is anesthetized with pentobarbital, placed in a stereotaxic apparatus, a burr hole drilled through its skull accoding to stereotaxic coordinates, and a commercially available microdialysis probe (CMA/10 probe of 3 mm length, 20 kD cutoff values; Carnegie Medicine, West lafayette, IN) will be lowered into the striatum. Artificial cerebrospinal fluid (CSF; 145 mM NaCl, 1.2 mM $CaCl_2$, 2.7 mM KCl, 1.0 mM $MgCl_2$, pH 7.4) is perfused slowly (1 μl/min) through the probe. Small molecules will diffuse between the artificial CSF and the extracellular fluid of the brain tissue. The rats are alowed to recover for 20-24 hrs, after which dialysate is collected from the effluent of the microdialysis probe. Dialysates (30 μl during 30 mins. intervals) are collected in polyethylene tubes containing 15 μl of EDTA-ethanol (0.02/1%) as a preservative at baseline and during administration of DA antagonists via the probe. The collected dialysates are stored at -70°C until subjected to HPLC analysis.

Analysis of monoamines and metabolites in tissue extracts and microdialysates

The frozen tissue extracts and microdialysates are thawed, and injected directly into a HPLC apparatus (Altex Ion Pair Ultrasphere C18, 4.6 mm inside diameter 250 mm Column No. 235335) coupled to an electrochemical detector (Model 460; Waters; Milford, MA) with an oxidation potential of 0.70 to 0.78 V. the mobile phase consists of 2 liters of water, 0.55 g of 1-heptanesulfonic acid, 0.2 g of EDTA, 16 ml of triethylamine, 12 ml of 85% phosphoric acid, and 80 ml of acetonitrile, pumped at 0.8 ml/min. In each sample subjected to HPLC, the levels of DA, as well as its metabolites dihydroxyphenylacetic acid (DOPAC) and hornovanillic acid (HVA) are determined by this procedure.

RESULTS

Example 12

PC12 cells were grown in culture as explained above, the cells were divided into three groups and different factors were added to the growth medium of each group for 48 hours as follows:

Group A - α glycerophosphate.

Group B - nerve growth factor (NGF) at a concentration of 50 ng/ml.

Group C – 1,3 cGP at a concentration of 1 μM.

The rate of neuronal growth in each of the above cultures was monitored and documented by microscopic photographs. As seen in Fig. 1, while growth of the cells in the presence of α glycerophosphate did not promote neural outgrowth in the cells (Fig. 1A) such neural outgrowth was clearly seen in the cells which were grown in the presence of NGF (Fig. 1B) or 1,3 cGP (Fig. 1C).

Example 13

Cells were grown as described in Example 12 above with the same factors and at distinct stages the level of intercellular signaling proteins were evaluated by a Western Blot technique using antibodies specific for the tested proteins.

Example 14

Cells were grown as described above and divided into groups which were each grown with one of the following:

(A)	growth medium	(B)	αGP	(C)	βGP
(D)	1,3 cGP	(E)	phenyl 1,3 cGP	(F)	1,2 cGP
(G)	1,3 cPP	(H)	phenyl-1,3 cPP	(I)	NGF

The above factors were added to the cells for a period of three hours after which they were washed away from the cells. The cells were further grown in a growth medium which did not comprise the above factors. The neural outgrowth of

the cells was monitored and microscopic photographs were taken on day seven after treatment with the above factors.

As seen in Fig. 2, under the above conditions, neural outgrowth was seen only in the cells incubated with the above CGs (Fig. 2D-2H). No neural outgrowth was seen in the cells incubated with the linear glycerophosphates (Fig. 2B and C) and under the above conditions NGF did not promote any nerve generation as well (Fig. 2I).

Example 15

PC12 cells were grown as described above and divided into the same groups as described in Example 14 above. The cells were incubated with the various factors for a consecutive period of seven days. Neural outgrowth was monitored and microscopic photographs were taken following the seven day incubation with the above factors.

As seen in Fig. 3, neural outgrowth was seen in cells incubated with the various above CGs(Fig. 3B-3H) as well as in cells grown in the presence of NGF (Fig. 3I) but not in the control cells grown with the linear glycerophosphates (Fig. 3A).

Example 16

PC12 cells are grown in culture in the presence of NGF under conditions allowing neuronal outgrowth of the cells. The NGF is then withdrawn by washing the cells and replacing their growth medium with a medium comprising no NGF. The cells then retract and the nerves disintegrate (analogous to the delayed neurodegeneration observed in the vicinity of injured nerves). Following this integration, the tested CG is added to the culture either for a short period of time after which it is washed or for a longer period of time and the CGs capability of *"rescuing"* the nerves is assessed by evaluating the re-differentiation of the cells into neuronal cells.

Example 17

Parkinson's disease is induced in rats as described in the Materials and Methods part above by injection of 6OH-DA into their brains.

The rats are then treated either with α and β linear GPs or with CG by administration of the either topically, per os, or directly into the brain using osmotic pumps as described above.

The effect of the linear GPs and of the CGs is assessed by evaluating the *in situ* production of L-DOPA, dopamine (DA), the dopamine metabolites DOPAC and HVA and the growth factor GDNF by using microdialysis techniques and by the methods described above. Motional and limb tremor parameters are also quantitatively evaluated in the rats treated with each of the above factors.

Example 18

Rats having injured optical nerves are treated with α and β linear glycerophosphates or with a CG as explained above and the effect of the above CG on the visual response and nerve generation of the treated rats is monitored.

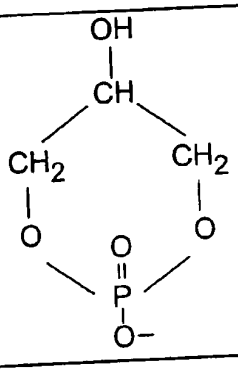
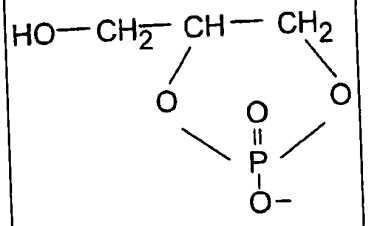
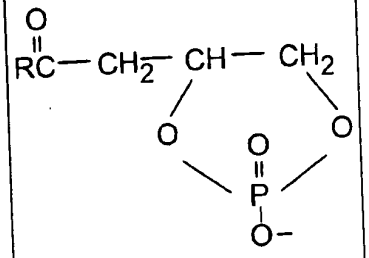
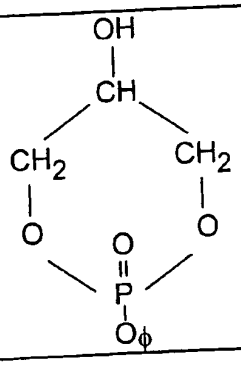
Example 19

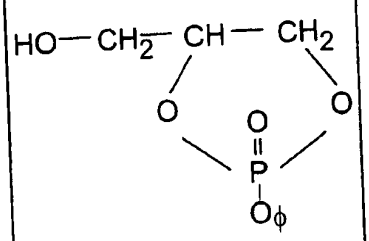
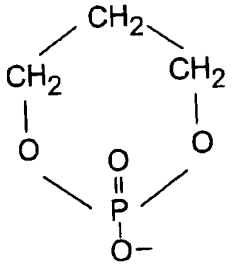
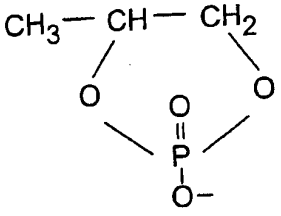
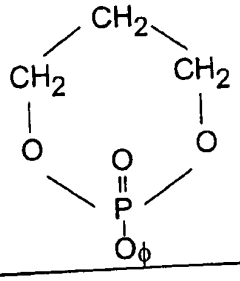
To study nerve rescue by 1,3 cPP, PC12 cells were incubated in tissue culture medium for a period of 14 days. Within this period, the cells were either grown in the presence of nerve growth factor (NGF) for different periods of times or were grown in the presence of 1,3, cPP for various periods of time. Neuronal differentiation and spread was examined in the various cells.

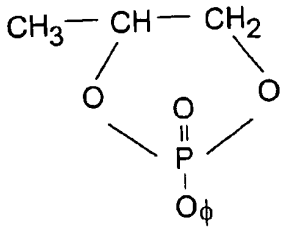
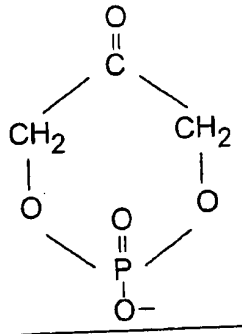
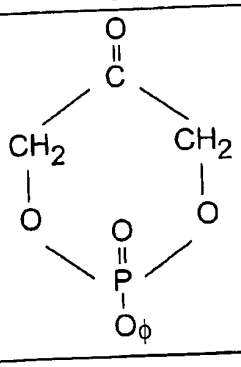
As seen in Fig. 4A, wherein the PC12 cells were grown in growth medium with no additives added, no neuronal spreading was observed (control). Growth of the cells in the presence of NGF (50 ng/ml) for the full period of 14 days resulted in full neuronal differentiation as seen in Fig. 4B. As seen in Fig. 4C, when the cells were grown for the first 7 days in the presence of NGF (50 ng/ml) and then cultured without NGF for an additional period of 7 days, complete nerve retraction was observed and the level of differentiation of the cells returned to control level.

Wherein the PC12 cells were grown for seven days in the presence of NGF (50 ng/ml) and for the remaining 7 days with either 2 μM, 4 μM or 6μM 1,3 cPP (Figs. 4D, 4E and 4F, respectively) partial to full rescue of the neuronal network (which developed during the first 7 days of incubation with NGF) from retraction was observed.

Appendix A

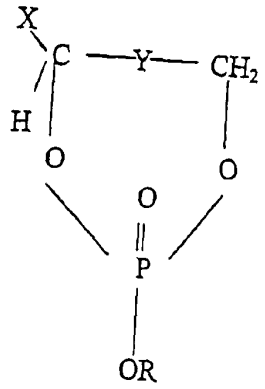
	Formula	Abbreviation
I		1,3 cGP
II		1,2 cGP
III		cyclic lysophosphatidic acid, c-lypoPA
IV		P-1,3 cGP

	Formula	Abbreviation
V		p-1,2 cGP
VI		1,3 cPP
VII		1.2 Cpp
VIII		P-1,3 cPP

	Formula	Abbreviation
IX		p-1,2 cPP
X		cDHAP
XI		P-cDHAP

CLAIMS:

1. A pharmaceutical composition for promoting neural cell differentiation in target cells comprising a pharmaceutically acceptable carrier and, as an active ingredient, a compound of the general formula I



wherein

Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl.

2. A pharmaceutical composition for promoting neural activity comprising a pharmaceutical acceptable carrier and, as an active ingredient, a compound of the general Formula I of Claim 1.

3. A pharmaceutical composition according to Claim 2, wherein said neural activity is selected from the group consisting of promotion of neuronal outgrowth, promotion of nerve growth, provision of dopaminotrophic supporting environment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue.

4. A pharmaceutical composition comprising a pharmaceutical acceptable carrier and, as an active ingredient, a compound of the general Formula I of Claim 1, for the prevention or treatment of disorders and diseases which can be prevented or treated by promoting neural cell differentiation and/or neural activity.

5. A pharmaceutical composition according to Claim 4, wherein said disorders and diseases are mental disorders.

6. A pharmaceutical composition according to Claim 5, wherein said mental disorder is schizophrenia or dementia.

7. A pharmaceutical composition according to Claim 5, wherein said mental disorder is a learning disability.

8. A pharmaceutical composition according to Claim 4, for the treatment of neurodegenerative conditions involving damage to the dopaminergic neural cells.

9. A pharmaceutical composition according to Claim 8, wherein said neurodegenerative condition is Alzheimer's disease.

10. A pharmaceutical composition according to Claim 8, wherein said neurodegenerative condition is Parkinson's disease.

11. A pharmaceutical composition according to Claim 4, wherein said disorders and diseases result from exposure to harmful environmental factors or from a mechanical injury.

12. A pharmaceutical composition according to Claim 4, for the treatment of nerve rescue after nerve injury.

13. A pharmaceutical composition according to any one of Claims 1-12, wherein the active ingredient is a compound of Formula I selected from the group consisting of:

i. 1,3 cyclic glycerophosphate - **1,3 cGP;**

ii. 1,2 cyclic glycerophosphate - **1,2 cGP;**

iii. 3-acyl 1,2 cyclic glycerophosphate (cyclic lysophosphatidic acid) - **c-lysoPA;**

iv. Phenyl 1,3 cGP – **P-1,3 cGP;**

v. Phenyl 1,2 cGP – **P-1,2 cGP;**

vi. 1,3 cyclic propanediol phosphate – **1,3 cPP;**

vii. 1,2 cyclic propanediol phosphate – **1,2 cPP;**

viii. Phenyl 1,3 cPP – **P-1,3 cPP;**

ix. Phenyl 1,2, cyclic propanediol phosphate – **P-1,2, cPP;**

x. Cyclic dihydroxyacetone phosphate – **cDHAP**; and

xi. Phenyl cyclic dihydroxyacetone phosphate - **P-cDHAP.**

14. A method for inducing promotion of neural cell differentiation of target cells comprising contacting said target cells for a suitable period of time with an effective amount of a compound of the general Formula I of Claim 1.

15. A method for promoting neural activity in an individual comprising administering to the individual in need an effective amount of a compound of the general Formula I of Claim 1.

16. A method according to Claim 15, wherein said neural activity is selected from the group consisting of promotion of neuronal outgrowth, promotion of nerve growth, provision of dopaminotrophic supporting environment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue.

17. A method for the prevention or treatment of disorders and diseases which can be prevented or treated by promoting neural cell differentiation and/or neural activity comprising administering to a person in need a therapeutically effective amount of a compound of Formula I of Claim 1.

18. A method according to Claim 17, wherein said disorders and diseases are mental disorders or diseases.

19. A method according to Claim 18, wherein said mental disorder or disease is schizophrenia or dementia.

20. A method according to Claim 18, wherein said mental disorder is a learning disability.

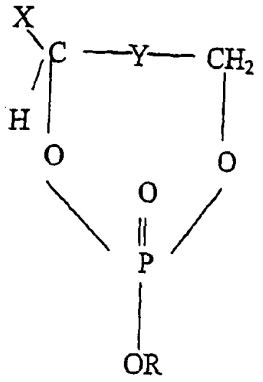
21. A method according to Claim 17, wherein said disorders and diseases are neurodegenerative disorders or diseases.

22. A method according to Claim 21, wherein said neurodegenerative disorder or disease is Alzheimer's disease or Parkinson's disease.

23. A method according to Claim 17, wherein said disorders or diseases result from exposure of an individual to harmful environmental factors or from a mechanical injury.

24. A method according to Claim 15, for the treatment of nerve rescue after nerve injury.

25. Use of a compound of the general Formula I



wherein

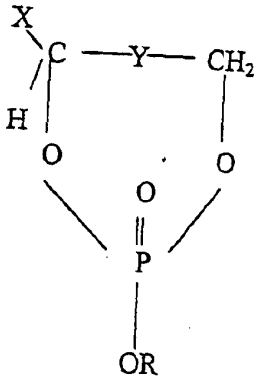
Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl

for the preparation of a pharmaceutical composition for promoting neural cell differentiation.

26. Use of a compound of the general Formula I



wherein

Y is $-(CH_2)_m-$, -CH(OH)- or -C(=O)- , and m is 0 - 3 ;

X is H, alkyl, $-CH_2OH-$, CH_2Oacyl or $-CH_2$acyl; and

R is H, a cation, alkyl or optionally substituted aryl for the preparation of a pharmaceutical composition for promoting neural activity.

27. Use according to Claim 26, wherein said neural activity is selected from the group consisting of promotion of neuronal outgrowth, promoting of nerve growth, provision of dopaminotrophic supporting environment in a diseased portion of the brain, prevention of nerve degeneration and nerve rescue.

28. Use according to Claim 25, for the prevention or treatment of disorders and diseases which can be prevented or treated by promoting neural cell differentiation and/or neural activity.

29. Use according to Claim 28, wherein said disorders and diseases are mental disorders or diseases.

30. Use according to Claim 29, wherein said mental disorder or disease is schizophrenia or dementia.

31. Use according to Claim 29, wherein said mental disorder is a learning disability.

32. Use according to Claim 28, wherein said disorders and diseases are neurodegenerative disorders or diseases.

33. Use according to Claim 32, wherein said neurodegenerative disorders or diseases are Alzheimer's disease or parkinson's disease.

34. Use according to Claim 28, wherein said disorders or diseases result from exposure of an individual to harmful environmental factors or from a mechanical injury.

35. Use according to Claim 27, for nerve rescue after nerve injury.

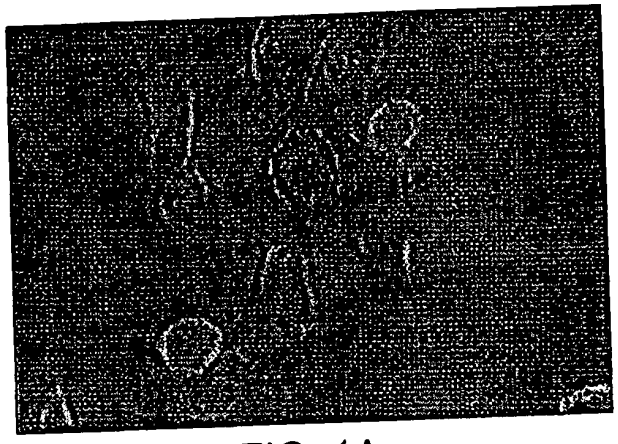


FIG. 1A

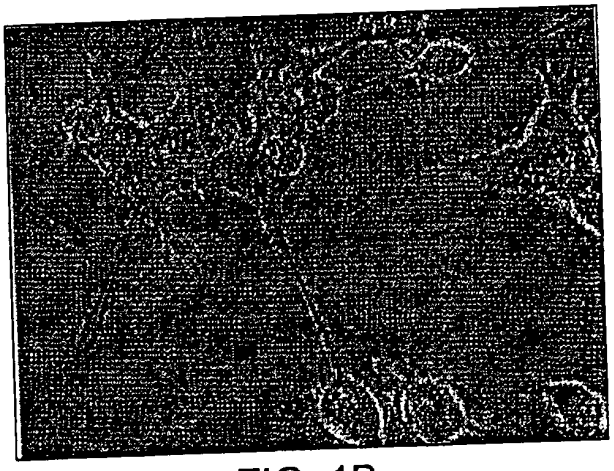


FIG. 1B

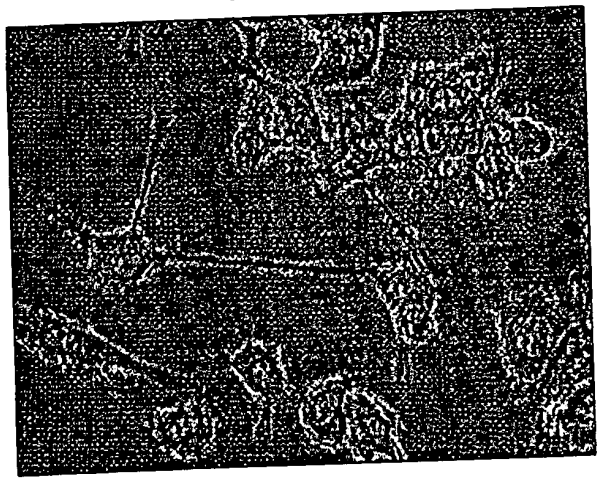


FIG. 1C

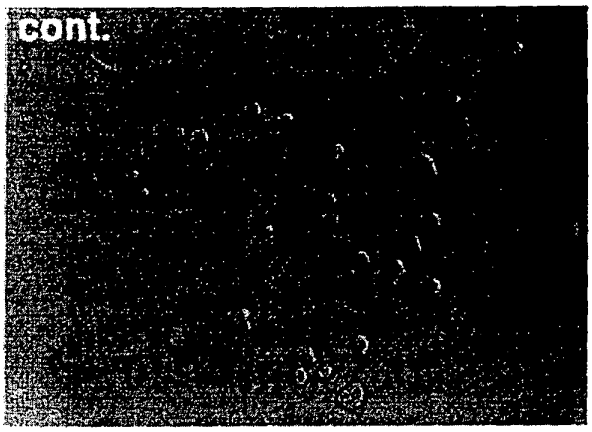


FIG. 2A

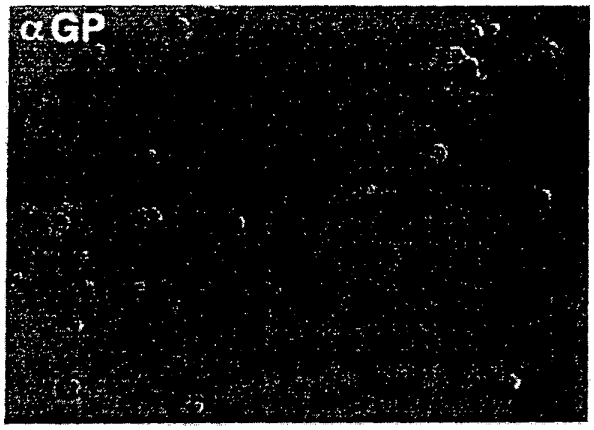


FIG. 2B

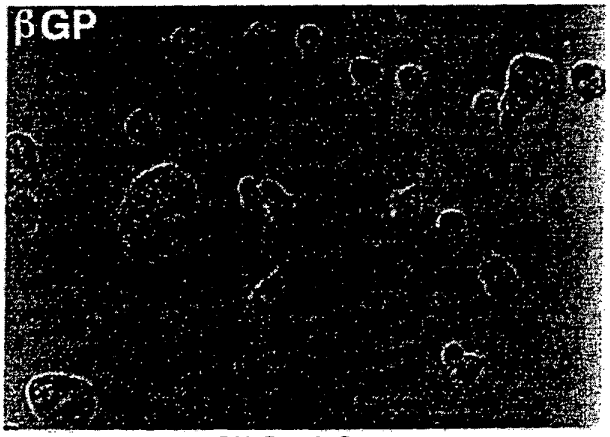


FIG. 2C

FIG. 2D

FIG. 2E

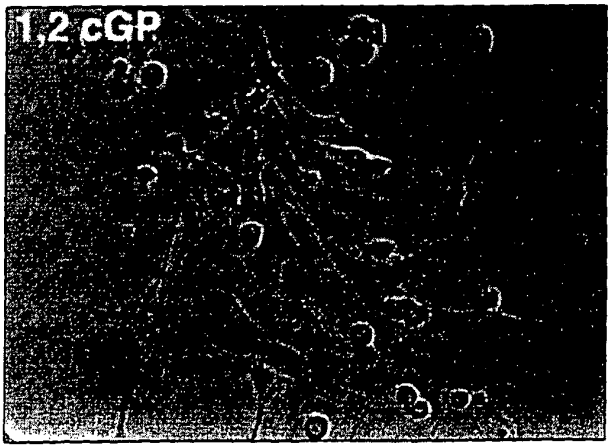


FIG. 2F

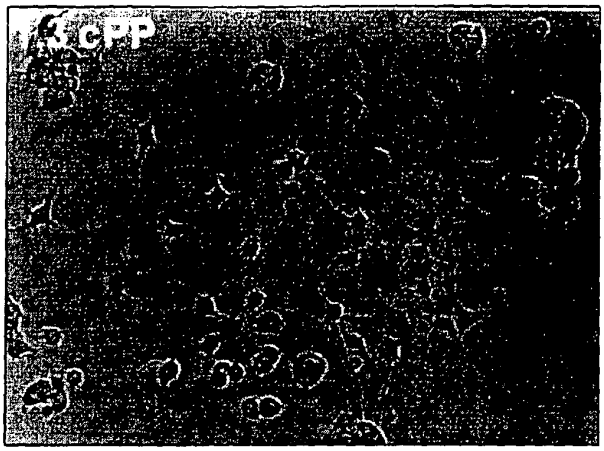


FIG. 2G

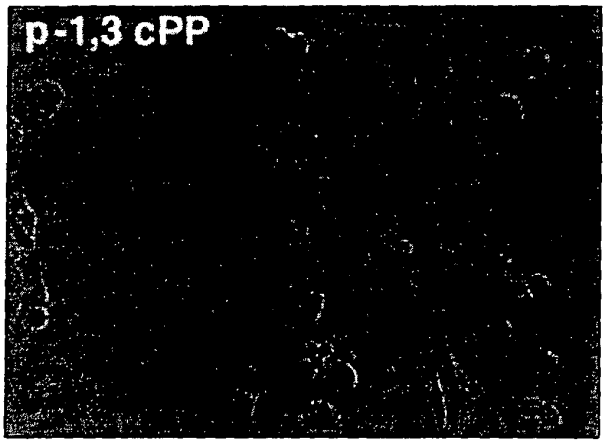


FIG. 2H

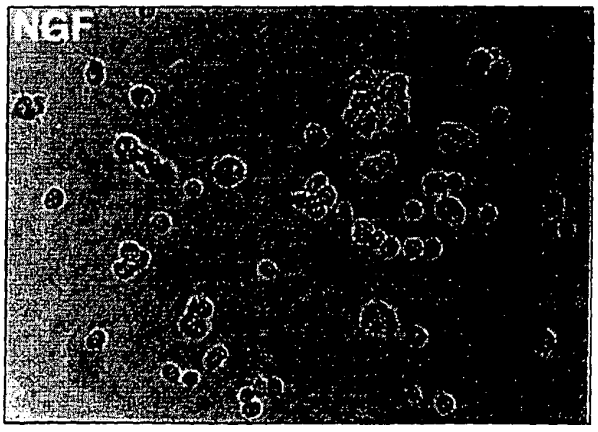


FIG. 2I

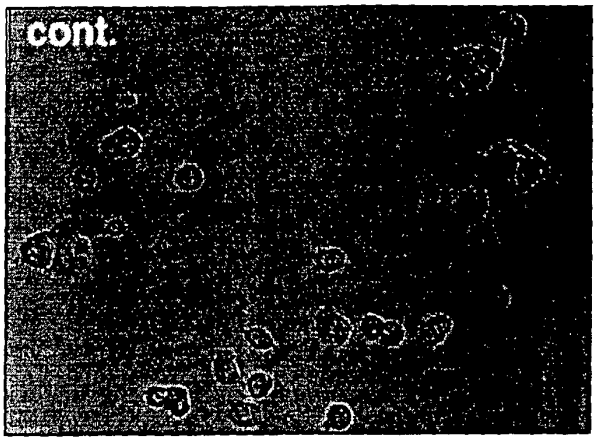


FIG. 3A

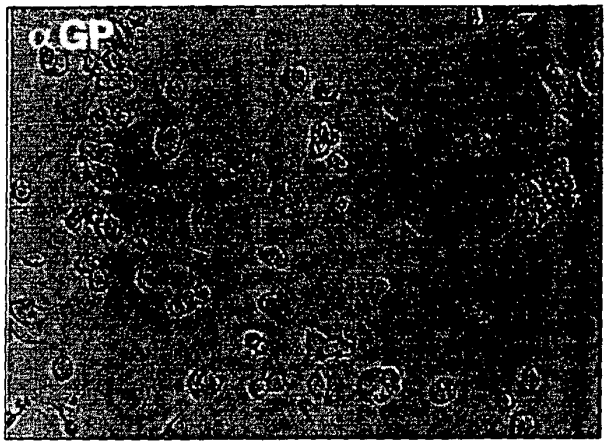


FIG. 3B

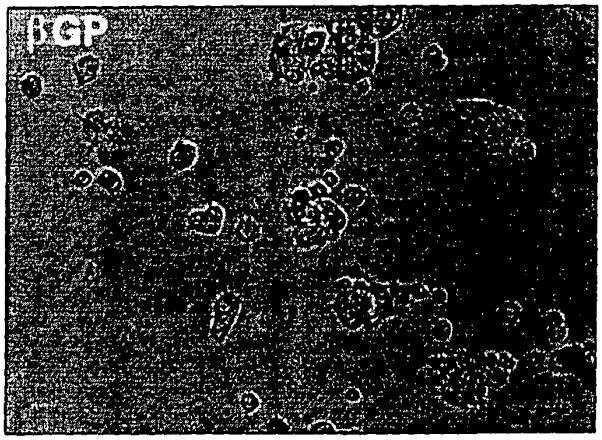


FIG. 3C

FIG. 3D

FIG. 3E

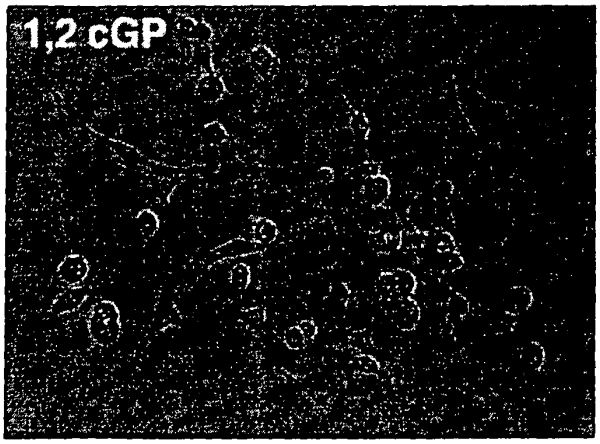


FIG. 3F

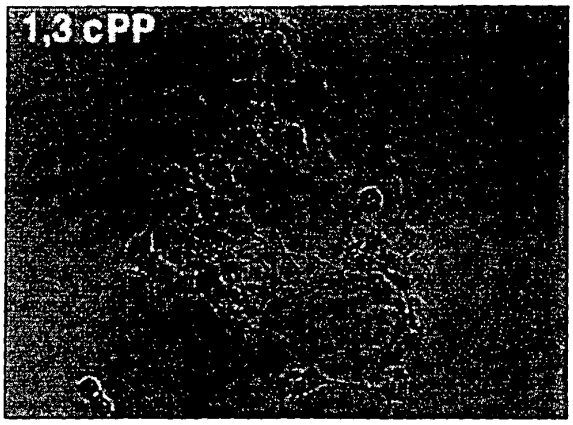


FIG. 3G

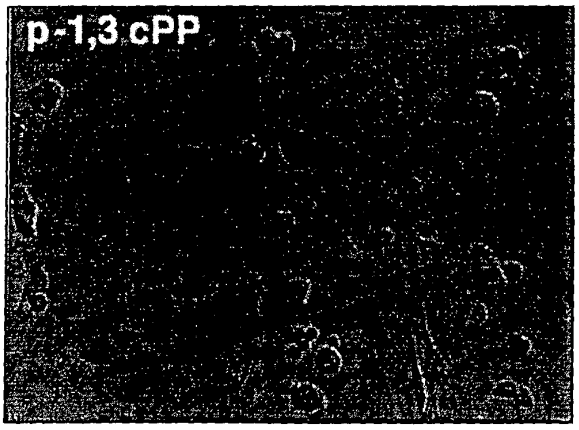


FIG. 3H

FIG. 3I

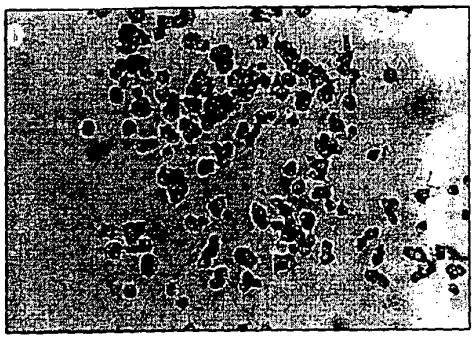


FIG. 4A

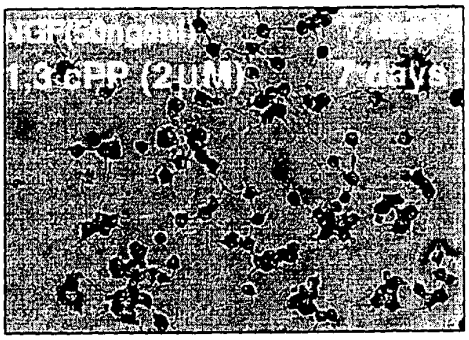


FIG. 4B

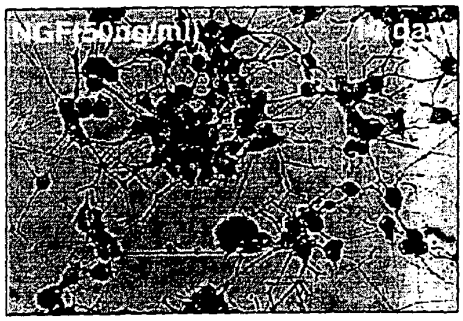


FIG. 4C

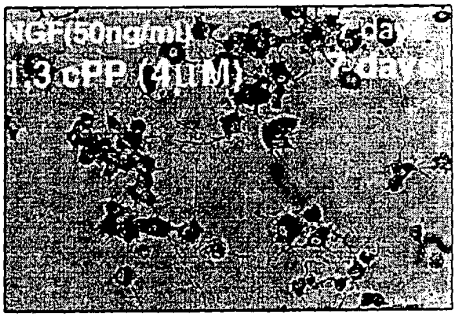


FIG. 4D

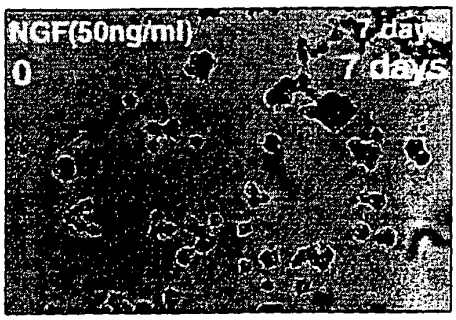


FIG. 4E

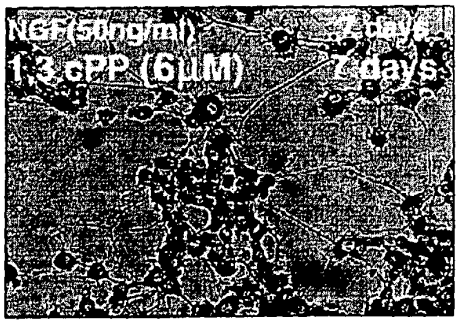


FIG. 4F

INTERNATIONAL SEARCH REPORT

Interr. ıal Application No
PCT/IL 00/00185

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/665 A61P43/00 A61P35/00 A61P35/02 A61P3/10
C07F9/6574

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, BIOSIS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	KINOR N ET AL: "Cyclic glycerophosphates for the treatment of Parkinson's disease" NEUROSCI. LETT., vol. 54, no. supp, November 1999 (1999-11), page S24 XP002155523 abstract ---	1-35
P,X	WO 00 09139 A (ALLELIX BIOPHARMA ;BEGLEITER LEATH E (CA); WICKENS PHILIP L (CA);) 24 February 2000 (2000-02-24) abstract page 11, line 11 -page 12, line 10 page 13, line 26 -page 14, line 22; claims; example 1 --- -/--	1-35

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
14 December 2000	28/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Orviz Diaz, P

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Form PCT/ISA/210 (second sheet) (July 1992)

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X	FRIEDMAN P ET AL: "CONVERSION OF LYSOPHOSPHOLIPIDS TO CYCLIC LYSOPHOSPHATIDIC ACID BY PHOSPHOLIPASE D" JOURNAL OF BIOLOGICAL CHEMISTRY,AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD,US, vol. 271, no. 2, 12 January 1996 (1996-01-12), pages 953-957, XP000946154 ISSN: 0021-9258 the whole document	1-13
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X	DATABASE CHEMABS 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOBAYASHI, SUSUMU ET AL: "Preparation of 1-O-acylglycerol-2,3-phosphates and DNA polymerase.alpha. inhibitors containing them" retrieved from STN Database accession no. 124:76506 XP002148571 abstract & JP 07 258278 A (SAGAMI CHEM RES, JAPAN) 9 October 1995 (1995-10-09) ---	1-13
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X	US 5 565 439 A (PIAZZA GARY A ET AL) 15 October 1996 (1996-10-15) abstract column 1, line 60 -column 2, line 39; claims; example II ---	1-13
X	D.C. AYRES ET AL.: "The Organic Chemistry of Phosphorus. Part V." J. CHEM. SOC., 1957, pages 1109-1114, XP000946300 see compounds (VI) and (VII) pages 1111 and 1114 --- -/--	1-13

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	REVEL, MONIQUE ET AL: "Phosphorus heterocycles. XXVII. NMR study of 4-monosubstituted 1,3,2-dioxa- and -dithiaphospholane derivatives" ORG. MAGN. RESON. (1976), 8(8), 399-406, XP000949315 page 399 ---	1-13
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